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Effect of hyperbaric oxygenation on myocardial function and levels of oxidative stress markers in rats with type I diabetes mellitus

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INTRODUCTION

Relevance of the research topic and the degree of its development

Diabetes mellitus (DM) is a group of heterogeneous diseases based on absolute or relative insulin deficiency. The most important are types 1 and 2 diabetes. Insulin-dependent type 1 diabetes (10-15% of the total number of patients) is caused by absolute insulin deficiency due to destruction of pancreatic β-cells. The disease usually develops by an autoimmune mechanism. The more common insulin-resistant type 2 DM is characterized by relative insulin deficiency caused by decreased tissue sensitivity to insulin or impaired insulin transport to them. Destruction of β-cells is not characteristic.

The main complications of chronic disturbance of metabolic processes in the body in DM are the development of diseases characterized by progressive dysfunction of all cellular structures, especially in vascular walls, heart, kidneys, visual organs, and nerves [30, 35, 87, 126].

DM affects more than 400 million people. This disease causes 1.6 million deaths per year [7]. The main target organs are the heart, kidneys, eyes, nerves, blood vessels, and brain [35, 87, 126], which can cause coma, macro-, microangiopathy, nephro-, neuro-, and ophthalmopathy, and other complications. The most frequent complication of DM is heart failure developing in 50% of patients, which leads to mortality in the first year after diagnosis in 30-40% of patients, and after 5 years this figure increases to 40-60% [7, 65, 131, 167].

Hyperbaric oxygenation (HBO) is a promising and obviously auxiliary method of diabetes treatment [11]. The impact of HBO on the clinical course of diabetes, especially type 1, is still poorly understood. The corresponding molecular mechanisms are even worse known. Meanwhile, it has been established that HBO improves recovery in such associated ischemic conditions as cerebral ischemia, combined ischemic and reperfusion injury, central retinal artery occlusion, peripheral arterial disease, diabetic foot gangrene and other vascular complications [11, 15, 16, 79, 142, 170, 171, 174, 176, 178]. HBO is able to reduce blood glucose concentration in type 2

DM patients by at least 20% [62, 118, 141, 178]. It also reduces the levels of HbA1c (a marker of inflammation), C-reactive protein and insulin resistance [62, 141]. It is assumed that one of the mechanisms of these effects is the attenuation of adipose tissue hypoxia and associated inflammatory reactions.

HBO improves microcirculation, enhances angiogenesis, activates antioxidant defense, stimulates fibroblast proliferation and collagen synthesis, leads to suppression of inflammatory processes, has antiatherogenic effect [78, 115]. HBO increases cell survival under hypoxia by improving mitochondrial respiration, inhibits apoptosis in pancreatic β-cells, disrupts leukocyte adhesion to the vascular wall, enhances their bactericidal action, and reduces the severity of endothelial dysfunction, which contributes to the subsidence of inflammation and reduces the development of associated vascular complications in patients suffering from DM [11, 168, 185].

The predictor of malignant arrhythmias in patients with type 1 and type 2 DM is the syndrome of prolonged QT interval. Daily HBO sessions for two weeks led to a decrease in prolonged QT interval [150]. The effect of HBO on diastolic myocardial function of patients with diabetes mellitus was studied by Aparci M. et al (2008). After 10 sessions of HBO the parameters of diastolic function according to Doppler-echocardiography improved. At the same time systolic and diastolic blood pressure, heart rate and echocardiographic parameters of the left ventricles did not change significantly. HBO also improved the relaxation capacity of the left ventricle and the dynamics of right ventricular filling [104]. It is assumed that HBO imitates ischemic preconditioning of myocardium with a corresponding decrease in the intensity of inflammatory reactions and oxidative stress.

Russian scientists have concluded that multiple courses of HBO are the most effective in patients with type 1 DM: the need for medicinal insulin is reduced, residual insulin secretion is restored, glucagon and hydrocortisone synthesis is suppressed. Three courses of HBO therapy administered to patients with type 1 DM with an interval of 4 months are more effective than two courses with an interval of 6 months; in this case, of the three procedures, the maximum effect is achieved during the second course. The same pattern is reproduced in type 2 DM [18, 19].

Intermittent HBO therapy enhances vascular relaxation induced by acetylcholine in rats with type 1 diabetes. The effect is realized through NO-signaling pathway without changes in oxidative stress indicators [109].

HBO in patients with type 1 DM can reduce the blood concentration of oxidative stress markers [18, 19, 101, 104, 109], which is one of the main factors damaging tissues [41]. At the same time, prolonged exposure to HBO can exacerbate oxidative stress and aggravate the aggressiveness of the disease course. Despite this, to date, there are no comprehensive studies that could evaluate the positive and toxic effects of HBO [5].

Purpose of the study

To reveal the effect of hyperbaric oxygenation, insulin therapy and their combined application on the indices of oxidative stress and pumping function of the heart, in experimental type 1 diabetes mellitus.

Research Objectives

1. To compare the hyperglycemic effect of oxygenobarotherapy, insulin therapy and their combined application in rats with type 1 diabetes mellitus;
2. to study the effect of hyperbaric oxygenation, insulin therapy and their combined effect on cardiodynamic parameters of isolated rat heart with type 1 diabetes mellitus;
3. To reveal the peculiarities of the effect of hyperbaric oxygenation, insulin therapy and their combined application on oxidative stress indicators (prooxidant markers) of the isolated rat heart with type 1 diabetes mellitus;
4. To reveal the peculiarities of the effect of hyperbaric oxygenation, insulin therapy, and their combined application on the indicators of oxidative stress (markers of prooxidants and antioxidants) in the blood of rats with type 1 diabetes mellitus;
5. Find potential molecular mechanisms of hyperbaric oxygenation action on blood glucose levels and cardiac performance in rats with type 1 diabetes mellitus.

Scientific novelty of the study

- A favorable and therapeutically effective effect of hyperbaric oxygenation on cardiodynamic parameters and myocardial contractility in rats with type 1 diabetes mellitus was shown for the first time.
- The positive effect of hyperbaric oxygenation on changes in coronary circulation in rats with type 1 diabetes mellitus was reported for the first time.
- It was revealed for the first time that hyperbaric oxygenation reduces in myocardial tissue of animals with type 1 diabetes mellitus the content of prooxidant factors: superoxide anion radical and hydrogen peroxide, initiating the process of lipoperoxidation, the products of which have a significant cytotoxic effect. This means that hyperbaric oxygenation has a significant antioxidant cardioprotective effect.
- For the first time it was registered that the use of hyperbaric oxygenation provided an increase in the efficiency of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). This allows us to recommend hyperbaric oxygenation to reduce the degree of cardiac alteration in type 1 diabetes mellitus.
- The therapeutic effect of hyperbaric oxygenation on the heart in rats with type 1 diabetes mellitus through the pharmacokinetic action of oxygen, oxygen-dependent response of myocytes (mitochondria) and homeostatic effect on the function of other organs was substantiated for the first time.

Theoretical and practical significance of the work

Functional indices of cardiac activity in type 1 diabetes mellitus improve after therapeutic HBO exposure. The molecular mechanisms of this effect are associated with activation of antioxidant system components and attenuation of oxidative stress. The combination of insulin therapy with HBO sessions causes an additive effect on the reduction of oxidative stress in type 1 DM, which may have clinical significance. The results of the study should be taken into account when using hyperbaric oxygenation as an adjuvant method of treatment of diabetes mellitus.

The actual data of the dissertation research and theoretical conclusions made on their basis are included in teaching materials (lectures, classes, etc.) and are used in teaching students, training residents, postgraduates and trainees at the Departments of Pathological Physiology of the Institute of Digital Biodesign and Modeling of Living Systems, as well as at the Department of Physiology, Faculty of Medicine, University of Kragujevac (Serbia).

Research methodology and methods

To study the effect of hyperbaric oxygenation on myocardial function and levels of markers of oxidative stress in rats with type I diabetes mellitus induced by streptozotocin injection, 24 healthy male Wistar albino rats aged 8 weeks with body weight of 200-250 g were used.

The experimental animals were divided into 4 groups of 6 animals each: those receiving insulin therapy, HBO treatment, combined treatment (insulin therapy together with HBO) and control group (untreated).

To monitor glycemia levels, experimental animals with type 1 diabetes mellitus were first subcutaneously injected with 5 units/day of exogenous long-acting recombinant human DNA insulin. To maintain normoglycemia (60 to 150 mg/dL), insulin therapy was given for 24 hours and then adjusted for each animal according to its glycemic level on average every 3 days (3 to 5 U/d).

HBO therapy protocol: experimental rats were exposed to 100% oxygen at 2.8 ATM once a day for 1 hour 5 days a week for 2 weeks.

Before withdrawal of the study animals from the experiment, they were injected with ketamine and xylazine, followed by decapitation using a guillotine for small experimental animals, immediately after which whole blood samples were taken and spectrophotometric determination of oxidative stress biomarkers and monitoring of cardiac function *ex vivo* using a Langendorff apparatus were performed.

The provisions of the prescribed acts (EU Directive for the Protection of the Vertebrate Animals used for Experimental and other Scientific Purposes 86/609 / EES) and the principles of ethics were observed in the experimental work.

Statistical processing of the obtained results was performed using the statistical package IBMSPSS version 26.0.

Main scientific results

Insulin monotherapy of experimental rats with insulin-dependent diabetes mellitus is accompanied by an increase in both pro-oxidants and antioxidants [11]. Quantitative changes in oxidative stress biomarkers showed that the levels of superoxide anion radical, hydrogen peroxide, malonic dialdehyde, nitric oxide in treatment groups 2, 3, 4 tend to decrease in comparison with group 1 of animals with diabetes mellitus. The most noticeable statistically significant decrease was observed in groups 3 and 4, where treatment was carried out with DM+INS+HBO and DM+HBO, respectively [5]. HBO promotes restoration of the initial activity of lipid peroxidation by changing the levels of the studied pro-oxidants (mainly due to their decrease) and increasing the levels of antioxidants (due to their increase), which agrees with the data of professional literature [5, 11]. HBO monotherapy reduces the content of superoxide anion radical and hydrogen peroxide, has no effect on the level of malonic dialdehyde and reduces the level of nitrogen dioxide, while activation of superoxide dismutase and catalase is observed, while the level of reduced glutathione is preserved [5].

In terms of diabetes compensation, repeated courses of HBO for a year reduce insulin consumption, restore residual insulin secretion, and inhibit the release of such counterinsular hormones as glucagon, somatotropic hormone and hydrocortisone. It is more effective to give patients with insulin-dependent diabetes three courses of HBO with an interval of four months than two courses with an interval of six months; however, with the three-course methodology, the maximum favorable effect of HBO on the hormonal-metabolic status is achieved only during the second course, and the third course only enhances the already achieved effect. Combined treatment with HBO and

exogenous DNA recombinant long-acting human insulin demonstrated not only a favorable effect on coronary oxidative status, but also a positive effect on cardiodynamics of the isolated rat heart. Systolic left ventricular pressure and the value of the maximum derivative of pressure over time are two cardiodynamic parameters characterising systolic function, and the parameters of the value of the minimum derivative of pressure over time and diastolic left ventricular pressure are related to diastolic function of the heart; all four of these cardiodynamic parameters as well as coronary blood flow improved in the group of rats receiving combined treatment (DM+INS+HBO) [71].

Author's personal contribution

The author is a key figure who participated in the choice of the research direction, formulation of its goal and objectives, as well as in the analysis and generalization of the obtained factual data. He was also involved in the preparation of the review of domestic and foreign literature on the research topic.

In the thesis, the author describes the laboratory research conducted, how the analysis and statistical processing of the obtained data was performed, summarizes, formulates and scientifically substantiates the results, provides conclusions emphasizing the theoretical and practical significance of the work and recommendations based on its results. Moreover, he was engaged in the preparation of scientific publications and reports, as well as their implementation in practice.

Provisions for defense

1. therapeutic HBO sessions improve indices of cardiac pumping function in experimental type 1 diabetes mellitus. This effect may be comparable with a similar effect of insulin therapy.

2. The combined use of insulin therapy and HBO most effectively improves cardiac functional parameters compared to each type of therapy separately.

3. therapeutic sessions of HBO, as well as insulin therapy can reduce excessively high activity of prooxidants, characteristic of type 1 diabetes mellitus. When HBO and insulin therapy are used together, an additive effect is possible.

4. Molecular mechanisms of improvement of functional cardiologic indices after sessions of HBO, insulin therapy and their combined application are associated with activation of antioxidant system with a corresponding decrease in the activity of pro-oxidants.

Compliance of the thesis with the passport of scientific specialty

Scientific provisions of the thesis correspond to the passport of specialty 3.3.3. - pathological physiology. The results of the conducted research correspond to the area of research of the specialty, specifically points 1 and 4 of the passport of the specialty.

Degree of reliability and approbation of the results of the thesis

The validity of the results of the thesis study was based on a representative sample of 24 rats divided into 4 groups of 6 individuals each. Statistical processing and comparative analysis of the collected data were performed as follows: 1) absolute values, their percentages, sample mean values, median and standard deviation, ranking and 95% confidence intervals were used to describe individual parameters; 2) Kolmogorov, Smirnov and Shapiro-Wilk tests were used to test the normality of distribution; 3) Student's t-test, paired t-test, Mann-Whitney test, Fisher's absolute probability test, and one- or two-factor analysis of variance were used to detect differences between the indicators. The Bonferroni test was used to test the significance of the difference between the parameters in the case of several subgroups. To assess the normality of distribution, Kruskal Wallis and Tukey post hoctest were used to compare percentage changes between groups. Differences at the level of $p < 0.05$ were considered significant. Statistical processing of the obtained results was performed using the statistical package IBMSPSS version 26.0.

The primary documentation of the dissertation was verified by the commission established by the order of the Vice-Rector for Research of FGAOU VO I.M. Sechenov First Moscow State Medical University of the Ministry of Health of Russia (Sechenov University), Associate Professor D.V. Butnaru, order of November 17, 2020, No. 270.

The materials of the thesis are used in the work of the Institute and the Department of Physiology of the Faculty of Medical Sciences of the University of Kragujevac (Serbia), the Department of Human Pathology of the I.M. Sechenov First Moscow State Medical University of the Ministry of Health of Russia (Sechenov University), as well as in the educational process at classes and lectures of the Department of Human Pathology and the Department of Pathophysiology of Sechenov University.

Approbation of the study results took place at a joint scientific and methodological conference of the staff of the Department of Pathological Physiology of the Institute of Digital Biodesign and Modeling of Complex Systems and the Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Serbia, which was held on November 26, 2020.

Publications on the subject of the thesis

On the subject of the dissertation 5 printed works were published, including:

- Scientific articles reflecting the main results of the thesis - 2 articles, of which:

- in publications from the University List/List of VAK under the Ministry of Education and Science - 1 article, in journals included in international databases: Scopus - 1 article;

- Review articles - 1 (in editions from the University List/List of VAK under the Ministry of Education and Science);

- Other publications include 2 abstracts.

Scope and structure of the thesis

The dissertation is outlined on _106_ pages of typewritten text, consists of introduction, literature review, description of materials and methods of research, research results and their discussion, conclusion. The list of literature contains _186_ sources, including _20_ domestic and _166_ foreign authors. The work is illustrated with _13_ figures and contains _3_ tables.

CHAPTER 1. LITERATURE REVIEW

1.1. Diabetes mellitus

1.1.1. Etiology and epidemiology of diabetes mellitus

Diabetes mellitus (DM) belongs to a group of heterogeneous diseases characterized by impaired carbohydrate metabolism, resulting in impaired tissue utilization of glucose, which causes an increase in its concentration in the blood. The main complications of chronic disturbance of metabolic processes in the body in DM are the development of diseases characterized by progressive dysfunction of all cellular structures, especially in vascular walls, heart, kidneys, visual organs and nerves [30, 87, 126]. According to the World Health Organization, 422 million people worldwide suffer from diabetes mellitus. Diabetes mellitus is directly responsible for 1.6 million deaths each year [7]. Statistics from the International Diabetes Federation are even more disappointing: as of 2021, there were 537 million patients with verified DM [120]. In addition to this, a huge number of patients have prediabetes. In the United States, for example, approximately 86.1 million adults are diagnosed with prediabetes. These data indicate a continuous global increase in the number of patients and prevalence of diabetes over the past several decades. Due to the fact that the complications of diabetes lead to damage to virtually all tissues in the body, it remains the leading cause of high mortality from cardiovascular disease (CVD), kidney failure, as well as disability of patients due to frequent infection of any wounds and circulatory failure, which provokes the need for amputation and complete loss of vision. In addition, early diagnosis of type 2 diabetes mellitus (DM2 or insulin-independent diabetes mellitus) in adolescents and young adults (under 40 years of age) is associated with a more aggressive form of the disease, as well as premature development of severe complications. This progressive increase in the prevalence of DM, especially DM2, is due to several reasons: aging of the population, sedentary lifestyle, poorly controlled glucose levels, smoking, and an increase in the number of obese patients. Obesity stands out as the most important risk

factor for the development of DM2, the level of which has increased significantly over the last few decades. However, it is known that this form of diabetes, in addition to obesity, is accompanied by impaired insulin secretion due to dysfunction of β - cells of the pancreas, which provokes the development of diabetes**.**

Impaired insulin secretion in DM may have different etiologies. According to the new guidelines, diabetes can be divided into several general categories:

1) Type 1 diabetes mellitus (DM1 or insulin-dependent diabetes), which is based on autoimmune destruction of pancreatic β-cells, which usually ends in the development of absolute insulin deficiency.

2) Type 2 diabetes mellitus (DM2 or insulin-independent diabetes) is characterized by impaired insulin secretion by β - cells, and is often accompanied by insulin resistance.

3) Gestational DM (first diagnosed during pregnancy in the second or third trimester, but not verified before pregnancy).

4) Specific types of DM of different etiologies, such as monogenic forms of diabetes (neonatal and juvenile diabetes, diseases of the exocrine part of the pancreas (e.g., cystic fibrosis or pancreatitis) and diabetes induced by drugs or chemicals (e.g., glucocorticoids, treatment of HIV infection or after organ transplantation) [30]. [30].

The clinical presentation and course of disease in DM1 and DM2 can be very different, demonstrating the heterogeneity of the disease itself. Adequate classification is crucial for determining therapy, but there are cases in which the disease cannot be clearly classified as DM1 or DM2 at diagnosis. Recently, the traditional view that DM1 is more common in children and DM2 in adults is not entirely accurate, given that both types of disease occur in different age groups [30]. In pediatric patients with DM1, symptoms such as polyuria and polydipsia are not uncommon, and in 1/3 of cases, diabetic ketoacidosis is an early complication of the disease [117]. At the same time, in adult patients at the debut of DM1, the symptomatology may present different manifestations: classical symptoms may be absent and even a temporary remission of the disease may develop [117, 182]. Therefore, the classification of DM is periodically reviewed and modified, because the variability of symptoms may lead to

misinterpretation of the type of diabetes and, accordingly, the establishment of an incorrect diagnosis leads to ineffective treatment. In all types of diabetes, the cause of hyperglycemia is the progressive death of pancreatic β-cells and / or impairment of their function, which occurs under the influence of various factors, both genetic and environmental factors. Therefore, patients with different types of DM often have the same chronic complications; the only difference may be in the rate of their progression [49].

Considering all these facts and the statistical data published to date, it is vital to identify the main causes of the development of DM and its complications in order to better and more effectively develop therapeutic intervention strategies for this disease.

1.1.2. Cardiovascular complications in diabetes mellitus

The fatal consequences of diabetes are the development of small blood vessel damage (microangiopathies), such as diabetic retinopathy, neuropathy, nephropathy; and macroangiopathies, as a result of which pathological changes affect large blood vessels. Macrovascular complications represent an aggressive course of atherosclerotic processes that significantly increase the risk of myocardial infarction, stroke, and gangrene of the foot (diabetic foot) [125].

As long-term studies by various specialized specialists have shown, heart failure occurs in 50% of all comorbidities in patients with diabetes. It is undeniable that patients with heart failure and diabetes have a worse prognosis and lower survival rate than those without it. Long-term clinical studies have revealed that about 30-40% of such patients die in the 1st year after diagnosis, and after 5 years this figure rises to 40-60%, which is usually caused by the occurrence of ventricular arrhythmias and refractory heart failure [65, 131]. The Chronic Heart Failure (CHF) classification - NYHA (New York Heart Association classification of CHF severity) provides useful prognostic information for each individual patient. According to the NYHA classification, the annual mortality rate is 30-70% in patients with stage IV, 5-10% in patients with stage II [28]. Interestingly, in people with a combination of DM and heart

failure, ejection fraction can be either preserved or reduced in an equal number of cases [169]. There is a direct relationship between the degree of metabolic disturbance in diabetes and the progression of CHF, so its development often begins already at the pre-diabetes stage. Therefore, the basic therapy of patients with diabetes and heart failure should include drugs to correct hyperglycemia and heart failure simultaneously. Treatment regimens for patients with heart failure and DM, blockade of RAAS (renin-angiotensin-aldosterone system), cardioselective blockade of β-adrenoreceptors, blockade of mineralocorticoid receptors, are the same as in patients without DM, although clinical results are not as favorable. The new angiotensin/neprilysin receptor inhibitors are thought to have a better effect on outcomes occurring in patients with DM and heart failure than without the former [37].

Arterial hypertension goes hand in hand with DM2. Thus, in patients with diabetes it occurs 2 times more often than without it. It has been recorded that patients with arterial hypertension are more likely to have insulin resistance than people with normal blood pressure, which makes them more likely to develop diabetes. CVDs complicated by the development of hypertension remain the leading cause of morbidity and high mortality in patients with DM. In the genesis of both diabetes and hypertension are traced common risk factors: the formation of endothelial dysfunction, the presence of inflammatory processes in the vascular wall, aggravated by arterial remodeling, the development of atherosclerosis, dyslipidemia and obesity. The same is traced at the stage of manifestation of cardiovascular complications in these nosologies [11, 125]. The most probable reason for such coincidences may be the unity of mechanisms of RAAS activation, oxidative stress, immune system, and inflammation development, which strengthens the link between DM and hypertension. Vascular mechanisms such as oxidative stress, inflammation, immune system activation, and microRNAs are found in both diseases. Thus, the vascular damage and endothelial dysfunction that forms in both DM and arterial hypertension exacerbate each other's effects [11, 140]. Therefore, the administration of hypoglycemic drugs and their effects are considered as an important clinical factor influencing blood pressure stabilization [11, 125]. Thiazolidinediones, which are PPAR-γ (peroxisome proliferator-activated receptor

gamma) agonists, have neutral or slightly positive effects on blood pressure [11, 43]. At this stage, there is no clear understanding of the following questions: what is the effect of drug correction of glycemic levels on the incidence of CVDs and what is the probability of a positive effect of adverse and adverse cardiovascular reactions that develop while taking hypoglycemic drugs on the benefit/risk ratio in the development of CVDs [84, 132]?

1.1.3. Diabetes mellitus treatment

Treatment of DM starts with changing the patient's habits and lifestyle, as most often its development is caused by poor nutrition of patients, and it is recommended to control body weight with increasing physical activity according to the general condition [7, 31, 120]. Next, the issue of treatment with medications is decided. At the stage of pharmacotherapy, the prescription of drugs should be gradual, taking into account individual reactivity, the effectiveness of glycemia correction, additional potential benefits, dosing regimen and economic feasibility of using certain drugs [7, 120, 83].

Since absolute insulin deficiency is observed in DM1, the leading method of its correction is insulin administration [7, 11, 83, 119, 120, 185]. If in DM2 oral hypoglycemic drugs are not able to normalize blood glucose and HbA1c (glycated hemoglobin or glycohemoglobin (A1c)) levels, both insulin monotherapy and combined with oral hypoglycemic agents' administration are possible [11]. Insulin therapy is most often started with the administration of basal insulin with a single daily dose of 0.1-0.2 J/kg body weight. In patients with higher episodes of hyperglycemia (HbA1c >9.0%), in addition to basal insulin, biphasic or prandial insulin can be administered before meals in single individual doses depending on the daily glycemic profile [45].

Metformin is the most prescribed and safe antidiabetic drug among the oral hypoglycemic agents of the biguanide group. It is used in obese and overweight people. Metformin is still the drug of choice for monotherapy because it increases tissue sensitivity to insulin, promotes glucose entry into peripheral tissue cells, and suppresses gluconeogenesis in the liver [129]. Metformin can promote weight loss and has been shown in some studies to reduce serum levels of triglycerides, cholesterol, and low-

density lipoprotein (LDL) [54]. After a certain time, metformin becomes an insufficiently effective pharmacologic agent in the processes of glycoregulation. All optional oral pharmacologic agents in combination with an optimal dose of metformin have approximately the same therapeutic effect, reducing HbA1c values by about 0.8-2.2% on average, but with different consequences for changes in body weight and susceptibility to hypoglycemic states [45, 155]. The combination of dual therapy with metformin and sulfonylurea derivatives is one of the most common. Together they contribute to more effective metabolic control and decrease HbA1c by 0.8-1.5%. Sulfonylurea derivatives enhance endogenous insulin secretion by pancreatic β-cells, but they have no long-term protective effect on β-cell function and may accelerate β-cell depletion and death [166].

Thiazolidinediones are peroxisome proliferator-activated receptor γ activators, which improves insulin sensitivity in adipocytes, cardiac muscle, and liver [63]. In insulin resistance in DM2 patients, the use of thiazolidinediones produces a persistent effect for up to 5 years [24]. A common side effect is weight gain. However, the greater the weight gain, the greater the reduction in HbA1c levels, as well as the restoration of β-cell function and insulin sensitivity [179]. The combination of metformin and one of the drugs from the group of thiazolidinediones leads to a significant decrease in HbA1c, an increase in the level of tissue sensitivity to insulin and, thus, leads to an improvement in the secretory role of pancreatic β-cells [159].

Dipeptidyl peptidase-4 inhibitors (DPP-4 or glyptins) act by inhibiting the enzyme of the same name. Inhibition of this enzyme is responsible for delaying the inactivation of incretin hormones such as glucagon-like peptide-1 (GLP-1) and gastroinhibitory peptide (GIP), which are involved in the physiological regulation of glucose homeostasis [163]. GLP-1 and GLP-2 induce pancreatic β-cells to synthesize insulin, and GLP-1 in isolation has the effect of reducing glucagon secretion by pancreatic α-cells [139]. These effects combine to improve glycemic control in people with DM2.

The use of GLP-1 analogs is mainly an incretin-based therapy. This leads to an increase in insulin secretion in a glucose-dependent manner, decreases glucagon secretion, and ultimately suppresses glucose production in the liver [55]. Persistent

reduction in HbA1c can be observed for up to three years. Although GLP-1 analogs are not as well tolerated by patients as DPP4 inhibitors, they lead to a better reduction in HbA1c levels and promote weight loss [165].

1.2. HYPERBARIC OXYGENATION

1.2.1. History of hyperbaric oxygenation application

The history of hyperbaric medicine dates to 1662, when British physician Nathaniel Henshaw built the first barobaric chamber. Unlike modern barochambers, the first chambers used compressed air instead of pure oxygen, as it was believed that oxygen had a toxic effect [82].

Oxygen was first discovered in 1775 by the English scientist John Priestley, and today this discovery is the basis for the use of hyperbaric therapy. In 1789, Lavoisier and Seguin reported the toxic effects of concentrated oxygen, thereby calling into question the use of oxygen in hyperbaric medicine. In 1878, Paul Burt more accurately described the toxic effects of oxygen on the central nervous system, manifested as seizures. Despite the view that excess oxygen is toxic, in 1887 Arnzenius analyzed the literature and recorded about 300 articles on the application of hyperbaric oxygenation. Thus, he showed that interest in the application of hyperbaric oxygenation (HBO) was expressed to a rather significant extent [86, 121, 122].

Several historic barocameras have been built during this time. The first barocamera in North America was created in 1860 in Canada. And the first chamber in the United States appeared in 1861 in New York City. The most famous and widely used barocamera in the USA was equipped for Cunningham in 1921, and by 1925 it became the only operating chamber in the world [52].

In the former Yugoslavia, the first barocamera was installed in 1933, but due to numerous technical problems it was never put into operation. At the UNA Navy Institute of Naval Medicine (Split, Croatia), a large recompression chamber for the

regular use of hyperbaric oxygen for clinical purposes became operational in 1969 [186].

The heyday of hyperbaric medicine dates to 1937: Benhke and Shaw first documented the success of treatment of decompression sickness with hyperbaric oxygen [67, 153]. Today, HBO is actively used in the treatment of many diseases, for example, in carbon monoxide poisoning, for the treatment of various infections, repair of injuries, including in patients with DM. The achievements of hyperbaric medicine are steadily growing, which expands the indications for the use of HBO [11, 162].

1.2.2. Definition and physiological principles of HBO

Hyperbaric oxygenation refers to therapeutic and diagnostic methods characterized by the creation of specialized conditions for the patient that allow inhalation of 100% pure oxygen at a pressure higher than atmospheric pressure. In practice, atmospheric pressure of more than 1.5 absolute atmospheres (ATM) (152 kPa) is usually used for therapeutic purposes. The following standard HBO prescription schemes are used: a person is under atmospheric pressure of 2.0-2.8 ATM (203-284 kPa) for 60-120 minutes once or twice a day. This allows reducing the risk of side effects from HBO while maximizing the therapeutic effect [11, 31, 38].

The effect of HBO on the body is related to gas laws and physiological and biochemical effects of hyperoxia itself [11, 102, 114]. The statement of Boyle's law, constant temperature, pressure, and gas volume are inversely proportional. This is the basis for many aspects of hyperbaric therapy, including a small increase in chamber temperature. During treatment, a phenomenon known as "squeezing" develops, arising because blocked eustachian tubes prevent the equalization of gas pressure, and this results in a painful gas compression response in the middle ear. Dalton's law expresses that in a mixed gas, each element exerts a pressure proportional to its fraction of the total volume (partial pressure) [183].

The effects of HBO are also based on Henry's Law: the amount of gas dissolved in a liquid or tissue is proportional to the partial pressure of that gas in contact with the

liquid or tissue. Henry's law can be applied to body fluids and oxygen: the degree of physically dissolved gas in body fluids is directly proportional to the partial pressure of oxygen to which that fluid is exposed. It follows that in proportion to the increase in the amount of oxygen to which the patient is exposed, the amount of dissolved gas increases, i.e. the oxygen content of the body fluids increases. In this case, the access of oxygen to tissues increases significantly, and it can diffuse from blood plasma, reaching ischemic tissues and each of their cells, regardless of the degree of pathological processes in the walls of blood vessels [8, 46, 183].

Oxygen delivery from the blood to cells is carried out in two ways: the first one consists in its transport in connection with hemoglobin in erythrocytes, the second one in the dissolved state in plasma. The effect of HBO is due to the displacement of gas bubbles from the plasma and an increase in the partial pressure of oxygen in the alveoli. This process leads to an increase in the volume of dissolved oxygen delivered by blood, which allows to increase its saturation of ischemic tissues. Thus, the increase in indices reflecting the amount of oxygen transported with blood contributes to the maintenance of cellular respiration and the possibility of adenosine triphosphate (ATP) formation in ischemic/hypoxic tissue [11]. It is proved that the main effects of HBO are related to its mechanical effect on tissues due to increased pressure and changes in the level of functioning of physiological systems of the organism [11, 90].

The biochemical aspect of HBO application is explained by activation of the mechanism responsible for the controlled formation of reactive oxygen species (ROS) and nitrogen species [11, 21, 29, 59, 76, 127, 160, 180, 181]. This results in an increase in the number of various growth factors and their receptors, activation of stem cells/bone marrow progenitor cells, functional changes in the effects of integrin (neutrophil adhesion is reduced) and chemokines synthesized by monocytes, as well as hemoxygenase-1, heat shock proteins and hypoxia-inducible factor 1-alpha (HIF-1), which leads to the subsidence of inflammatory processes [11].

Thus, HBO contributes to the leveling of negative consequences of pathological processes in which inflammation and ischemia take the lead, which is observed, for example, in chronic wounds [76, 162,183] (Figure 1).

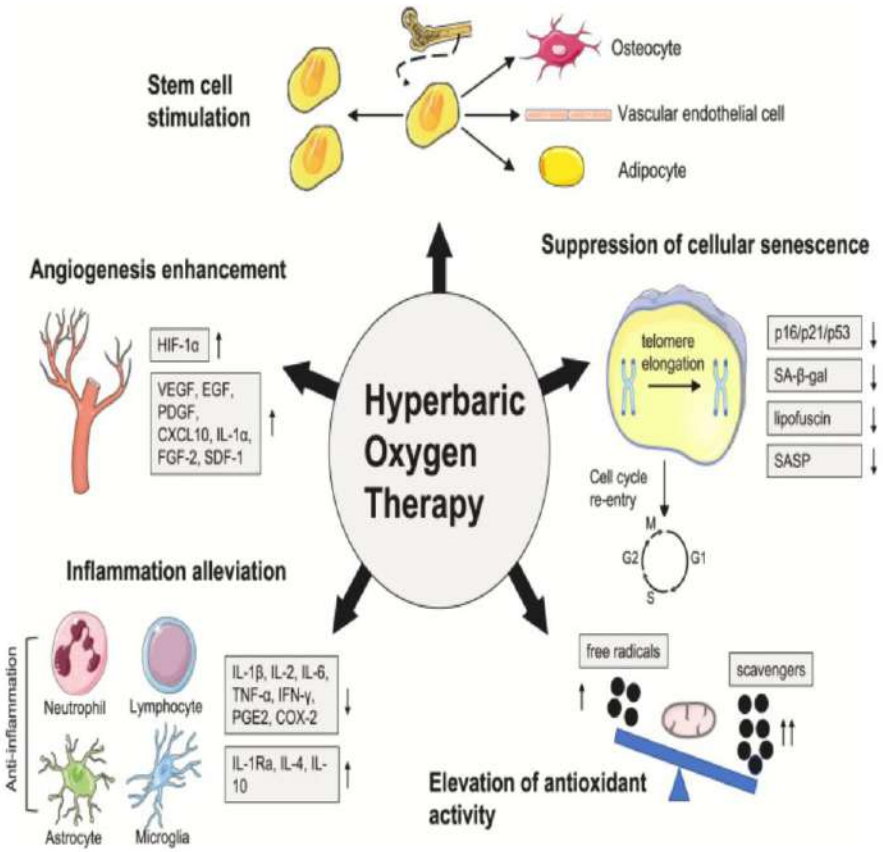

Figure 1 - Biochemical basis of hyperbaric oxygenation

1.2.3. Indications for the use of hyperbaric oxygenation

Originally, hyperbaric therapy was developed to treat decompression sickness and the side effects of diving. Today, however, the benefits of HBO allow it to be used as a primary or adjunctive treatment for many internal diseases and surgical conditions. Since the 1960s, the list of indications for HBO has been expanding. According to the 2016 data of the Undersea and Hyperbaric Medical Society (UHMS), the following diseases and syndromes with different etiopathogenesis are indications for the use of HBO [11, 76]:

- the toxic effects of carbon monoxide;
- anemia;
- gas embolism;
- decompression sickness;

- to improve wound repair (in diabetic foot, diabetic retinopathy, nephropathy);
- intracranial abscess;
- osteomyelitis;
- necrotic soft tissue infections;
- gas gangrene;
- chronic radiation lesions;
- severe multiple traumas with ischemia;
-burn;
- skin graft transplantation [11].

The use of HBO in acute air embolism and decompression sickness is that compressed oxygen bubbles, which occur at elevated atmospheric pressure, displace emboli with nitrogen and equalize the pressure gradient between the lungs and the bloodstream, facilitating embolus utilization. In addition, HBO can benefit a variety of conditions, including those mentioned on the list above, through a similar underlying mechanism. For decades, the mechanism of action of HBO has been aimed at leveling developing or evolved tissue hypoxia by increasing physically dissolved oxygen in blood plasma. It is known that an increase in atmospheric pressure with inhalation of 100% oxygen leads to an increase in the amount of oxygen dissolved in plasma. For example, a study published in 1960 by Boerem et al. showed that unanesthetized pigs with 0.5% circulating hemoglobin could live because of a significant amount of dissolved oxygen in plasma when hyperbaric conditions were created.

The application of oxygen more than the physiologic norm can cause various both therapeutic and toxic effects [11, 64, 97, 98, 107, 148, 178]. Potential therapeutic effects include hydrostatic pressure effects accompanied by elimination of inert gases from tissues, pharmacological effects due to increased oxygen pressure in arterial blood, and improvement of oxygen saturation of ischemic tissues with different degrees of functional impairment [11, 38].

Positive results from the use of HBO compared to absorption of atmospheric oxygen are due to a higher efficiency of oxygen penetration through the alveolar barrier, which leads to a higher concentration of oxygen dissolved in the blood and an increased

distance for its delivery. These properties make it possible to retain aerobic metabolism in various parts of the body even in tissues with hypoperfusion [11, 177].

The basis for the development of HBO treatment protocols is the essence of the main pathological processes causing the development of the disease. For example, in the presence of diabetic wounds, HBO application is carried out at a pressure of 2.0-2.4 ATM for 30-40 days [63]. In case of strokes, it is recommended to carry out HBO treatment for 2 months with a regime of 5 days a week and pressure of 2 ATM [96, 105].

1.2.4. Contraindications to the use of hyperbaric oxygenation

The only absolute contraindication for the use of HBO in the treatment of patients is untreated pneumothorax. If in the patient's history there is evidence of treatment due to the development of pneumothorax, considering all the indications, it is necessary to make an assessment between the risks and possible benefits obtained by using HBO [80].

Relative contraindications to the use of HBO include:

- Untreated arterial hypertension;
- Diabetes mellitus (with glycemic levels greater than 300 mg% or less than 100 mg%);
- Congestive heart failure with ejection fraction less than 35%;
- Claustrophobia;
- Acute upper respiratory tract infection;
- Presence of fever;
- Presence of a pacemaker;
- Recently undergone ophthalmic surgery;
- Recently performed thoracic surgery;
- Presence of chronic obstructive bronchitis or asthma;
- Constant use of contact lenses [11, 80].

1.2.5. Side effects of hyperbaric oxygenation

HBO is the safest among all treatment modalities for DM [61, 108]. Nevertheless, there are side effects associated with the use of HBO that are self-limiting and potentially preventable with adequate risk assessment. The most common side effects of HBO are barotrauma to the middle ear and sinuses, increased systolic and diastolic blood pressure, claustrophobia, and the development of hypoglycemic reactions in patients with DM [108, 118]. Toxic effects of oxygen during HBO application are manifested by symptoms of central nervous system dysfunction, development of arterial gas embolism or pulmonary barotrauma [88, 148, 151, 161]. Adherence to clinical treatment recommendations when using HBO under conditions of atmospheric pressure maintenance at the level of 2-3 ATM rarely provokes their occurrence [108].

Side effects caused by exposure increased atmospheric pressure and/or increased oxygen concentration led to excessive formation of ROS in organs and tissues. HBO application at pressures of 2026-3039 hPa increases the risk of cataract and myopia formation [11, 23, 80, 95, 106, 138]. Some authors, exposing guinea pigs to hyperbaric oxygen at a pressure of 2534 hPa for 2-2.5 h twice a week for a total of up to 100 sessions, recorded the development of cataracts in experimental animals. It was also shown that HBO depletes the pool of reduced glutathione in the lens nucleus, provokes oxidative changes in nuclear proteins, nuclear light scattering and myopic shift of lens power, which resembles the course of pathological processes preceding the formation of cataract in humans [11, 23]. HBO has an opposite effect on lens geometry and gradient refractive index compared to similar effects recorded after ouabain-induced inhibition of the microcirculatory system. In contrast to the ouabain-induced shifts in the indices of lens geometry and increase in the gradient refractive index and myopic shift, HBO does not lead to a significant transformation of lens geometry, although it significantly reduces the gradient refractive index, causing a decrease in lens power and the development of a hyperopic shift in the general optics of the bovine lens [11, 95].

Similarly, myopia and cataracts have been diagnosed in people after exposure to 100% oxygen at a pressure of 2026-2534 hPa for 90 minutes once a day for 150-850

sessions [138]. These pathologies rarely develop when 48 sessions of HBO at a pressure of 2534 hPa for 90 minutes are applied [81].

HBO promotes an increase in the number of inflammatory cells in mice [11, 77, 181] and leads to excessive production of ROS in rats [11, 72, 136, 147, 183], rabbits [11, 175], and humans [11, 42, 107]. Excessive ROS production is a key in the mechanisms of formation of many diseases and their complications. Intensification of oxidative stress and increased free radical levels trigger pathological mechanisms responsible for the development of atherosclerosis, myocardial infarction, arterial hypertension, diabetes, cataracts, retinopathy, renal failure, and uremia [11, 39, 85, 89, 128, 130, 157].

During therapy with 40% oxygen and more, regardless of atmospheric pressure parameters, multiple side effects caused by damage of erythrocytes by formed ROS and decrease in the amount of oxyhemoglobin are registered in rats [137]. The mild effect of HBO, observed at oxygen content up to 35-40%, does not cause aggressive course of oxidative stress in humans [73] and rats [56].

When HBO is used to treat chronic coronary heart disease, a potential undesirable effect may be the development of pulmonary edema. Weaver and Churchill in their study recorded 3 cases of pulmonary edema after HBO treatment. All three patients were elderly and had foot or chest wounds. In addition to the primary indications, the patients were also diagnosed with heart disease and low ejection fraction. Therefore, the authors suggest to try to refrain from using HBO in the treatment of patients with heart failure and reduced ejection fraction [184].

In a study conducted by Canarslan-Demir K. et al. who studied the cardiovascular effects of HBO treatment in patients suffering from DM, according to ECG data at the end of hyperbaric oxygenation session a statistically significant increase in QTc and QTc dispersion ($p<0,001$ and $p = 0.02$, respectively) and an increase in troponin I level, while the dynamics of Pro-BNP level did not show statistical reliability ($p = 0.009$, $p = 0.3$, respectively) [11, 34]. Thus, the authors concluded that in patients with DM, HBO treatment with short courses has a statistically significant effect on troponin I, QT and QTc indices, therefore, monitoring of the corresponding parameters is necessary in the group of such patients when using HBO [34].

In one of the scientific publications there was a report on the development of acute pulmonary edema in an 80-year-old patient with ischemic cardiomyopathy (with an ejection fraction of 25%), insulin-independent diabetes mellitus and peripheral vascular disease after HBO. Heart failure was not documented in the patient prior to HBO sessions. The authors of the publication believe that the risk factor for the development of acute pulmonary edema caused by HBO exposure was the total high sensitivity of the patient's tissues to hypoxia [11, 26].

There is also a paper describing a case of HBO-induced pulmonary edema in a patient diagnosed with moderate diastolic dysfunction with normal ejection fraction [11]. In this example, the assumed mechanism of HBO-induced pulmonary edema formation is an increase in cardiac afterload, increased left ventricular filling pressure, increased myocardial oxidative stress, increased pulmonary capillary permeability or pulmonary oxygen toxicity. Based on the above, the authors recommend using HBO with caution in patients with low ejection fraction or diastolic dysfunction [147].

To reduce the risk of pulmonary edema associated with the use of HBO, profile specialists suggest individualizing the mode and dosage of HBO sessions.

1.3. Hyperbaric oxygenation and the cardiovascular system

Among all the most important chronic diseases as well as causes of sudden death, CVDs occupy the most important place [68, 135]. They account for 45% of all deaths in Europe. Each year, CVDs cause more than 4 million deaths in Europe and about 1.8 million deaths in the European Union [68]. The term CVD is used to refer to conditions affecting the heart and/or blood vessels but can also be associated with arterial damage in the heart as well as in the brain, kidneys and eyes [135]. The main pathologies responsible for the development of CVD include atherosclerosis, arterial hypertension, coronary artery disease and CVD [11].

Even though at this stage there is a huge range of drugs available for the treatment of these diseases, the long-term prognosis and control of complications remain at a rather low level. Considering the rapid population growth, the contingent of patients receiving

inadequate therapy is increasing, which is a strong incentive for the creation of new, more effective methods of treatment and prevention of CVD development [11, 164].

Among the therapeutic methods of treatment, HBO is more often beginning to be used as a universal treatment. To ensure the effectiveness and safety of HBO treatment, the correct dosage plays a vital role [154].

It has been shown that HBO application in patients with chronic ischemic heart disease after 12-15 sessions significantly reduces and even stops the occurrence of angina pectoris, alleviates symptoms of heart failure, and significantly improves myocardial contractility [9, 17, 20]. Also, continuous HBO treatment of 31 patients with paroxysmal tachycardias in ischemic heart disease significantly reduced the frequency and duration of paroxysms with subsequent long-term remission [10]. The use of HBO has a significant effect on reducing the number of extrasystoles, as well as on improving myocardial contractility in patients with chronic ischemic heart disease [13, 20].

Two randomized trials studied the oxygen effect of HBO in patients with myocardial infarction receiving thrombolysis. Contradictory results were obtained. In one study, which was conducted on 74 patients with first-time acute myocardial infarction, it was recorded that additional HBO after thrombolysis in acute myocardial infarction has a favorable effect on left ventricular systolic function and remodeling process. In patients who received combined treatment with streptokinase and HBO, a 20% decrease in end-systolic volume and a 10% improvement in cardiac output were observed. In the same group, a significant increase in ejection fraction was demonstrated, while in the group of patients receiving streptokinase alone, a decrease in ejection fraction was observed three weeks after myocardial infarction [27]. With another study HOT (Hypertension Optimal Treatment study), which included 112 patients, it was shown that the use of HBO leads to shorter periods of pain relief, a slight increase in ejection fraction without a significant decrease in the level of creatine phosphokinase. However, oxygen therapy also did not affect the diastolic function of the left ventricle [94].

Leitman et al (2020) conducted a prospective study to evaluate the effect of HBO on echocardiographic parameters in asymptomatic patients. To study the effect on cardiac function, HBO treatment was applied for 90 minutes for a total of 60 days. The study

recorded an improvement in left and right ventricular systolic contractions as well as overall cardiac function. Because repeat echocardiography was performed 3 weeks after the last hyperbaric oxygen therapy session, these significant changes represent potentially sustained structural and functional changes rather than immediate temporary changes [173].

A clinical study by Yogaratnam et al. (2010) showed that preconditioning of patients with ischemic heart disease using HBO before cardiac surgery improved ejection fraction and reduced myocardial damage [97]. The results of the study by Zhdanov and Sokolov also indicate the beneficial effect of HBO in combination with conventional therapy of myocardial infarction. Their combined treatment effectively eliminated hypoxia and improved myocardial contractile function, as well as its pumping function [14].

Animal studies have also shown the benefits of HBO use. In the study conducted on the model of rats with induced myocardial infarction, HBO administration caused a decrease in the infarct size and an increase in the survival rate of rats [32, 111]. Higher values of total antioxidant response and 3-nitrotyrosine in the zone of tissue damage of the left heart sections in rats on HBO indicate higher production of nitrogen monoxide (NO), which contributes to better recovery in the ischemia zone and better vascularization [53].

Positive effects of HBO were also observed in the doxorubicin-induced cardiotoxicity model in rats. Application of 100% oxygen at a pressure of 2.5 atmospheres for 90 minutes did not enhance the toxic effects of doxorubicin. Moreover, the authors concluded that HBO treatment protects myocardial structures from doxorubicin-induced toxicity [98].

Regarding the effect of HBO therapy on ROS formation Tepic et al. (2018) concluded that HBO therapy may be safe when used in DM2 patients with and without vascular complications. They find that ten-day HBO treatment of 1 hour duration strongly mobilizes the antioxidant enzyme system and thus improves protection against oxidative damage [116].

On the other hand, during acute single exposure to HBO, superoxide production increases and, therefore, there is a temporary impairment of vasorelaxation. Intermittent HBO has no effect on superoxide production, but the expression of antioxidant enzymes increases under such exposure [25].

Oliveira et al. (2020) on the model of acute myocardial infarction studied changes in the redox system associated with HBO therapy occurring within the first hour after the development of coronary occlusion. HBO treatment for 60 minutes at a pressure of 2.5 atmospheres was shown to reduce mortality, which in turn indicates a favorable effect of HBO on survival. Since no differences in the size of myocardial infarction were observed, the authors concluded that the decrease in animal mortality could be due to a beneficial effect on the change of infarction-induced redox processes, which are important for maintaining a favorable environment for cell survival and heart rhythm [111].

Chen W. et al. (2020), who studied in rats with ligated left anterior descending artery the ways of activation of protective sanogenetic mechanisms under the influence of HBO in terms of changes in the level of mitochondrial functioning and triggering autophagy, showed how exactly hyperbaric oxygen has a positive effect on heart function: there is a decrease in the level of oxygen stress, modulation of energy metabolism and inhibition of cell apoptosis. The authors also showed how HBO application prevents cardiomyocyte damage in the simultaneous coexistence of ischemic and reperfusion processes in the altered myocardium: by increasing the levels of ATP, ADP, energy charge and opening the mitochondrial permeability transition pore, mitochondrial dysfunction is prevented, autophagy is inhibited, and gene or protein expression levels of eIF4E-binding protein 1 are increased, target of rapamycin (mTOR), mitochondrial DNA, NADH dehydrogenase subunit 1, mitofusin 1 and mitofusin 2, decreased levels of AMP, gene or protein expression of autophagy 5 (Atg5), dynamin-related protein 1 and p53, cytochrome c and reactive oxygen species [99].

Oliveira et al. conducted an additional study on rats to investigate the effect of early HBO on mortality and myocardial infarction size after the formation of coronary

occlusion in them, which showed mixed results. In this work, the authors recorded that HBO administration significantly reduced infarct size, but no significant change in mortality was observed. A possible explanation for the discrepancy between the results of these studies may be a difference in the protocol of HBO treatment itself [111].

1.4. Hyperbaric oxygenation and diabetes mellitus

The criteria for prescription and positive effects of HBO therapy in many pathological conditions, such as decompression sickness and DM2 in the presence of poorly regenerating wounds and ulcers, are well defined. Disturbance of oxygen balance in blood plasma occupies an important place in the pathogenesis of DM. Unfortunately, insulin administration does not always allow adequate daily maintenance of blood glucose levels within the normal range [11]. Therefore, it is desirable for patients suffering from DM to join additional methods of DM treatment, such as HBO therapy. HBO improves recovery in such concomitant DM ischemic conditions as cerebral ischemia, combined ischemic and reperfusion injury, central retinal artery occlusion, peripheral arterial disease, diabetic foot gangrene and other vascular complications [11, 61, 103, 106, 142, 178].

There is evidence of additional benefits of HBO in patients with DM, such as reducing fasting blood glucose concentration in DM2 patients by at least 20% [22, 62, 102, 118, 142]. In addition, HBO can cause a decrease in HbA1c, a marker of inflammation, C-reactive protein, and insulin resistance, as measured by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [142]. However, the data on the effect of HBO on the sensitivity of peripheral tissues to insulin are insufficient. It is not quite clear whether the hyperbaric oxygen-induced decrease in glycemia is a phenomenon unique to diabetics or whether it is really a physiological change that people with DM2 cannot compensate for. Thus, when studying the effect of HBO therapy on the change of tissue tolerance to insulin in male patients aged 40-80 years, suffering from DM2 and obesity or without them, with verified diabetic ulcers, osteoradionecrosis, and radiation proctitis, an improvement of peripheral tissue

sensitivity to insulin was revealed, which came already after 3 sessions and persisted up to the 30th [11, 185]. It should be noted that insulin therapy was carried out in all patients, and three of them were additionally prescribed oral hypoglycemic drugs. During HBO-therapy oxygen delivery is under increased pressure and in high concentration, at the same time an increase in tissue sensitivity to insulin is registered, which can be caused by the effect of either one of these factors or their combination. Under the action of HBO, the increase of oxygen delivery with blood to tissues is amplified 10 times. This suggests that one of the ways to realize the effect of HBO on increasing tissue sensitivity to insulin may be a decrease in the severity of hypoxia in adipose tissue and the following inflammation. It has also been shown how hypoxia in adipose develops overstress in the endoplasmic reticulum and chronic microinflammation, which leads to inhibition of the insulin signaling cascade [11, 92, 158]. In addition, some research results indicate a decrease in the level of C-reactive protein after HBO application [11, 51, 142], which can potentially be interpreted as the possibility of controlling the processes responsible for the maintenance of chronic microinflammation with HBO. Another explanation for the increase in peripheral tissue sensitivity to insulin during HBO exposure may be the increased expression of PPAR-1α, which is the main regulator of mitochondrial biogenesis, which was demonstrated in one of the studies conducted on rats [57]. Recent studies [93, 123] confirmed the beneficial effect of HBO on tissue sensitivity to insulin and at the same time laid the foundation for possibly new potential mechanisms of this effect. They proved that in mice with streptozotocin-induced DM2 after HBO treatment (1 h/day, 7 days, at 2.0 ATM) the activation of Akt signaling pathway, the expression of GLUT4 (glucose transporter member 4) in muscle and UCP1 (uncoupling protein 1, thermogenin) in brown adipose tissue, which subsequently leads to attenuation of insulin resistance and reduction of glycemia [93, 3].

By triggering various molecular mechanisms in the body, HBO realizes its beneficial therapeutic effect on patients with diabetes and its vascular complications. In studies by different authors, it was demonstrated how HBO positively affects the systemic hemodynamic response: microcirculation improves, angiogenesis is stimulated,

antioxidant defense is activated, fibroblast proliferation and collagen synthesis are stimulated, inflammatory processes subside, the intensity of atherosclerotic vascular damage decreases and the area of atherosclerotic plaques decreases [11]. After exposure to hyperbaric oxygen there is an increase in its partial pressure for 4-6 hours after each session. As a result, there is a restoration in mitochondria of an adequate level of biochemical reactions related to oxidative phosphorylation and an increase in the chances of cell survival under conditions of oxygen deficiency. There is also a blockade of apoptosis activation mechanisms in pancreatic β-cells and decrease of leukocyte adhesion, increase of their bactericidal action, and decrease of endothelial cell dysfunction severity. Thus, inflammatory processes subside, which is reflected in the minimization of diabetic vascular complications in patients [11, 172, 185].

NO occupies a special position in the process of carbohydrate metabolism disturbance, causing the formation of DM. In this case, the change in NO concentration during HBO therapy enhances its effectiveness in DM. The mechanism of NO synthesis realization is important. It is known that the development of vascular complications in DM is caused by activation of abnormal NO synthesis, which is one of the characteristic features of chronic hyperglycemia. The data obtained by different researchers studying changes in different ways of NO synthesis activation under the influence of HBO on DM background are too heterogeneous. For example, HBO, stimulating the formation of endothelial nitric oxide synthase (eNOS) leads to an increase in the accumulation of nitric oxide in wounds, triggering enhanced angiogenesis [11,40, 44, 110]. And the analysis of the results obtained in human studies indicates a decrease in nitric oxide synthesis under the influence of HBO due to the changes in the regulation of activation of inducible and neuronal nitric oxide synthases (iNOS, nNOS), reflected in the subsidence of inflammatory processes and a decrease in the progression of atherosclerotic vascular lesions [11, 36, 58, 100, 116, 133].

After confirming in healthy rats, the positive effect of HBO on the reactivity of isolated aortic rings to angiotensin-(1-7) [11, 60], a significant role of eicosatrienoic acids (EETs) in this process was revealed. The confirmation of the obtained conclusions was made after the experiment on rats with streptozotocin-induced DM1 [11, 113]:

aortic relaxation under the action of angiotensin-(1-7) (ANG-(1-7)) is registered much higher in rats with DM1 exposed to HBO than in rats of the control group (with DM1 without HBO). At the same time, no data on the pronounced effect of angiotensin II on aortic contraction under HBO were obtained. The mechanisms of the facilitated vasodilatory effect of ANG-(1-7) caused by HBO exposure, which led to such results, may be both the influence of EET (against the background of increased vascular sensitivity) and the increased expression of some isoforms of CYP enzyme (isoenzyme of cytochrome P450 family): mRNA of CYP2J3 and CYP2C11. HBO leads to the development of a pronounced, enhanced vascular response to ANG-(1-7) formation in DM1 rats. At the same time, no significant change of ANG-(1-7) concentration in serum was observed, because of which the authors concluded that after HBO application the changes in vascular reactivity are not a consequence of possible changes in blood pressure, the amount of ANG-(1-7) in blood or increase of oxidative stress level [11, 60]. Perhaps, this effect explains the positive effect of HBO in such pathological conditions as, for example, healing of chronic diabetic ulcers. The same authors, having conducted a study of angiotensin-converting enzyme (ACE) activity after HBO therapy, came to similar conclusions regarding ACE activity in serum: after HBO treatment in healthy rats, in contrast to rats with diabetes, there is an increase in ACE concentration [11, 124].

Many researchers pay attention to the identification of prolonged QT interval (PQTI) syndrome, recognized as one of the main factors in the development of malignant arrhythmias and lethal outcome [11, 144], which significantly worsens mortality rates in patients with diabetes. PQTI is more frequently diagnosed in patients with DM than without it [11, 75, 91]. Therefore, a clinical study was conducted to investigate the effect of HBO therapy on patients with DM1 or DM2 and QT SUI. After two years of follow-up of patients with PQTI, it was significantly shorter in patients receiving HBO compared to patients in the placebo group. This indicates a pronounced protective effect of HBO against QT interval prolongation and the resulting high risk of adverse outcomes in the diabetic patient population [74]. The mechanism for the higher prevalence of PQTI in diabetic patients is not completely clear, but potential reasons

could be a higher incidence of myocardial infarction as well as the presence of cardiac autonomic neuropathy, factors that are known to be associated with the occurrence of PQTI [47, 150, 151]. In addition, the PQTI syndrome is known to prolong during hypoglycemia [74]. Similar results were obtained by scientists [34, 101] who studied the effect of HBO on the dispersion of the QT interval (QTd) as an indicator of heterogeneity of excitability recovery, which is considered by profile specialists as a predictor of the development of malignant arrhythmias, especially in patients suffering from DM2. It was demonstrated how repeated HBO application for 2 weeks decreases QTd in patients with DM [11, 101, 150].

According to one of the new concepts of cardiac dysfunction formation in DM, it is believed that the development of diastolic myocardial dysfunction precedes systolic dysfunction, regardless of the influence of other factors, such as arterial hypertension, ischemic heart disease, etc. This significantly changes the morbidity and mortality rates of patients with DM. This significantly alters the morbidity and mortality of patients with DM. Left ventricular diastolic dysfunction is considered as one of the early predictors of unfavorable prognosis in patients with DM even though it is detected even under adequate metabolic control [11, 143, 156]. The effect of HBO on the diastolic function of the left ventricle in patients with diabetes was studied by Aparci et al. (2008). They proved that after 10 sessions of HBO the parameters of myocardial diastolic function measured by Doppler-echocardiography improved, while systolic and diastolic blood pressure, heart rate and echocardiographic parameters of the left ventricles did not change significantly. HBO therapy also resulted in improvement of left ventricular relaxation capacity and right ventricular filling dynamics [104]. It is assumed that the mechanism of such a favorable effect on the myocardium lies in the fact that HBO imitates ischemic preconditioning of the myocardium, thus leading to a decrease in inflammation and oxidative stress [32, 145].

More than 20 years ago there were observed advantages, but also certain differences in the effects of HBO therapy in DM1 and DM2. Our researchers came to the following conclusion: HBO treatment of patients with DM1 for a year with several courses gives a more pronounced positive effect manifested by reduction of insulin dose, restoration of

its residual secretion and suppression of secretion of such hormones as glucagon, growth hormone, and hydrocortisone than a single course of treatment. It was found that treatment of patients with DM1 with HBO in three courses with a periodicity of 4 months leads to a more pronounced positive effect than two courses with a break of 6 months. Interestingly, the maximum positive effect of hyperbaric oxygen on the hormonal-metabolic status occurs already at the second procedure, and the third one only enhances the achieved effect. The same modality was observed in DM2 [18, 19].

Recently, the effect of intermittent HBO therapy on acetylcholine-induced vasorelaxation in female Sprague-Dawley rats with diabetes was investigated in an isolated aortic model. Acetylcholine-induced vasorelaxation was examined *in vitro* on aortic rings, and systemic oxidative stress, plasma antioxidant capacity, and expression of various genes were evaluated to determine the possible mechanism. It is believed that HBO improves acetylcholine-induced vasorelaxation through NO-signaling pathway without affecting oxidative stress [109].

CHAPTER 2. MATERIALS AND METHODS OF RESEARCH

2.1. Materials and methods

To study the effect of hyperbaric oxygenation on myocardial function and levels of oxidative stress markers in rats with type I diabetes mellitus, 24 healthy male Wistar albino rats aged 8 weeks with a body weight of 200-250 g were used. The rats were kept in a vivarium with alternating light and dark periods every 12 hours at +22 ± 2°C. Experimental animals received water and standard feed.

Before taking the study animals out of the experiment, they were injected with ketamine and xylazine, after which decapitation was performed using a guillotine for small experimental animals, immediately after which whole blood samples were taken. The tubes, thoroughly mixed with 3.8% sodium citrate solution, were placed in a centrifuge, and spun at room temperature 3 times for 10 minutes each at 3000 rpm [5]. To prepare hemolysate, the obtained mass was dissolved in distilled water.

The provisions of the prescribed acts (EU Directive for the Protection of the Vertebrate Animals used for Experimental and other Scientific Purposes 86/609 / EES) and the principles of ethics were observed in the experimental work.

The animals were randomly divided into 4 experimental groups:

Group 1 (control) - DM provoked by streptozotocin (STZ) administration (n=6);

Group 2 - DM+INS, in which NPH (neutral protamine Hagedorn) insulin therapy at 3-5 units/day was performed against the background of STZ-induced DM. (n=6);

Group 3 - DM+INS+HBO (in addition to similar conditions for group 2, animals were exposed to 100% oxygen at 2.8 ATM 1 h once a day for 5 days for two weeks (n=6);

Group 4 - DM+HBO (laboratory rats with STZ-induced DM were exposed to HBO according to the scheme used in Group 3) (n=6) [5].

Modeling of type 1 diabetes mellitus

Twenty-four hours before intraperitoneal injection of STZ (60 mg/kg body weight dissolved in 0.01 M sodium citrate buffer at pH=4.5) into experimental rats to model DM1, they were not fed. Verification of DM was performed using a portable glucometer that reflected blood glucose levels obtained from tail veins [5]. Confirmation of the developed DM1 in experimental animals was the excess of blood glucose over 11.1 mmol/l.

Protocol for treatment with human NPH insulin

To monitor the glycemia level, exogenous DNA recombinant *long-acting* human insulin (RNA insulin) was injected subcutaneously into experimental animals with DM1 [5, 146]. To maintain normoglycemia (60 to 150 mg/dL), insulin therapy was administered for 24 hours. The initial dose of NPH insulin, which was 5 units/day, was adjusted on average every 3 days for each animal depending on its glycemic level and ranged from 3 to 5 units/day [5].

Hyperbaric oxygenation

To expose experimental rats to hyperbaric oxygen, they were placed in a small animal barocamera in which they were exposed to 100% oxygen at 2.8 ATM once a day for 1 hour [2] (Figure 2).

Protocol: experimental animals received HBO therapy 5 days a week for 2 weeks.

Figure 2 - Special oxygenation chamber for small experimental animals.

Spectrophotometric determination of biomarkers of redox status

To study the effects of hyperbaric oxygenation on myocardial function and levels of markers of oxidative stress in rats with type I diabetes mellitus, levels of superoxide anion radical (O_{2-}) were determined in coronary venous outflow samples, hydrogen peroxide (H_2O_2), lipid peroxidation index (LPI) measured as TBARS, nitrite (NO_{2-}), reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). All biochemical analyses were performed spectrophotometrically (Shimadzu UV-1800UV-VIS spectrophotometer, Japan) [5].

Determination of the superoxide anion radical

Determination of the amount of O_{2-} in coronary venous outflow and plasma is based on the reaction of O_{2-} with Nitro Blue Tetrazolium (NBT) to form nitroformase blue. The measurement is performed at a wavelength using λ_{max} =550 nm [5, 48, 12].

Reagents

Reagents used to determine O^2 levels: TRIS (Tris (hydroxymethyl) aminomethane, $C_4H_{11}NO_3$), 37% hydrochloric acid (HCl), Na_2EDTA, nitro-tetrazolium blue chloride (NBT, $C_{40}H_{30}ClN_{10}O_6$), gelatin ($C_{15}H_{11}N_2NaO_2$).

Protocol

50 µl of sample and 950 units of "assay mixture" were added to a test tube (12x100). The "assay mixture" was a mixture of 50 mmol Tris-HCl buffer (pH=8.6), 0.1 mm EDTA, 0.1 mg/mL gelatin, and 0.1 mm NBT. Krebs-Henseleit solution was used as a control. At the beginning of the reaction, the extinction of the mixture was measured, and this was labeled as extinction E1. The extinction was then stirred with a plastic stick every 60 seconds and the extinction after stirring was recorded, and this process was repeated until two consecutive extinctions reached approximately the same value [12]. The last extinction was denoted as E2. The same procedure was carried out for the control. The concentration of released O_{2-} was measured.

The amount of released O_{2-} per gram of cardiac tissue was then determined using the following equation [12]:

nmol O_{2-} /minute/g = $\Delta E/0.015$ x 1/0.05 x CF/m $_{heart}$

Determination of hydrogen peroxide

The determination of H_2O_2 is based on the oxidation of phenolic red by a hydrogen peroxide reaction catalyzed by the enzyme Horser Radish PerOxidase (HRPO) [12]. Using this method, the occurrence and release of H_2O_2 within a time interval of 5-60 minutes can be determined [138].

Reagents

The reagents used to determine H_2O_2 levels are potassium dihydrogen phosphate dihydrate (K_2HPO_4 x $2H_2O$), potassium dihydrogen phosphate dihydrate (KH_2PO_4 x $2H_2O$), sodium chloride (NaCl), hydrogen peroxide (H_2O_2), D(+)-glucose monohydrate (dextrose), phenol red ($C_{19}H_{14}O_5S$), horseradish peroxidase (HRPO EC 1.11.1.7).

Protocol

200 μL of coronary venous outflow tract and 800 μL of freshly prepared Phenol Red Solution-PRS were added to tubes (12x100) (Phenol Red Solution-PRS). PRS was prepared by mixing 140 mmol of NaCl, 10 mmol of potassium phosphate buffer (pH =7), 5.5 mmol of D(+)-glucose, and 0.28 mmol of phenol red. Then 10 μl (1: 20) of HRPO which was prepared ex tempore was added to the samples. The samples were left at room temperature for 10 minutes, after which a pH of ≈12 was adjusted using 1M NaOH. A solution of KC instead of coronary venous outflow tract was used as a control [12].

Absorbance (A) was measured spectrophotometrically at the wavelength of maximum absorbance λ_{max} =610 nm, in glass civets with a volume of 1 mL. The absorbance value of the control absorbance (B) was subtracted from the absorbances obtained, giving the final absorbance (ΔA). The concentration and then the amount of H_2O_2 released in the coronary venous outflow tract was calculated based on [12]:

1) absorption coefficient (F)
2) of the sample absorber
3) amount of H_2O_2 released per gram of heart tissue.

Determination of lipid peroxidation index (measured as TBARS)

The lipid peroxidation index was determined indirectly by measuring the products of the reaction of lipid peroxidation with thiobarbituric acid, i.e. TBARS (Thiobarbituric

Acid Reactive Substances) levels. The spectrophotometric method is based on the determination of lipid peroxide levels based on the reaction of malonyl dialdehyde (MDA) with thiobarbituric acid (TBA) [134, 12].

Reagents

The reagents used to determine TBARS levels were 2-thiobarbituric acid (TBA), 28% trichlorosyrucetic acid (TCA), and sodium hydroxide (NaOH).

Protocol

800 μL of coronary venous outflow tract sample and 200 μL of 1% TBA in 0.05 M sodium hydroxide were placed in a test tube. The samples were incubated on a water bath for 15 minutes at 100^{o} C followed by incubation at room temperature for 15 minutes before absorbance determination. Absorbance was measured spectrophotometrically at ⌊=530 nm. The Krebs-Henseleit solution was used as a control in an amount equivalent to the coronary venous outflow volume [12].

The amount of TBARS released per gram of cardiac tissue was calculated as follows:

nmol TBARS/minute/g weight = $\Delta A/1.56 \times 1.25 \times CF/m_{heart}$

TBARS levels were expressed within μmol, unlike other measured prooxidants whose concentrations are expressed in nmol, because the amount of TBARS is one rank higher than all other parameters [12].

Determination of nitrite

Measurement of levels of released NO_2^- in coronary venous outflow represents a suitable method to indirectly assess the functionality of the endothelial L-arginine: NO system in the coronary circulation.

Nitric oxide (NO) reacts with molecular oxygen to form nitrite (NO_2^-) as follows:

$NO + ½O_2 \rightarrow NO_{2-}$

Because equimolar amounts of nitrite are formed in this reaction, it is believed that the level of nitrite in the coronary venous outflow tract is the level of nitric oxide released. The method is based on the use of Griess reagent, which in the presence of nitrite leads to the formation of a violet-colored diazocomplex [33, 12].

Reagents

Reagents used to determine NO_{2-} levels: Sulfanilic acid (4-aminobenzulfonic acid), N-(1-naphthyl)-ethylenediamine dihydrohydrate (NEDA), ammonium chloride (NH_4Cl), borax ($Na_2B_4O_7$, 10h2o), 85% orthophosphoric acid (H_3PO_4), ⟩=1,685 gcm^{-3} ; sodium nitrite ($NaNO_2$) [12].

Protocol

The concentration of released nitrite was determined based on a calibration curve, the construction of which was based on the extinction of samples containing a known concentration of nitrite after reaction with Griess reagent in the presence of buffer [12]. By adding 3, 6, 12 and 24 µl of aqueous solution of 1 mmol $NaNO_2$ in 1 ml of KH solution, obtaining the desired nitrite concentration. The next step was to determine the standard factor (F) and divide the difference of extinction of the sample and control by the standard F:

nmol NO_2 /mL outflow = ΔE (E_u-E_{sp})/F

The amount of nitrite released per gram of cardiac tissue was then determined as follows [12]:

nmol NO_2 /minute/g weight = $\Delta E/F$ x CF (coronary flow)/m_{heart}

Determination of reduced glutathione

Determination of antioxidant defense enzyme - reduced glutathione (GSH) activity was measured in erythrocyte lysate by spectrophotometric method. This method is

based on the oxidation reaction of glutathione by 5, 5-dithio-bis-6, 2-nitrobenzoic acid using the Beutler method. To determine the concentration of reduced glutathione, 200 μl of 0.1% ethylenediaminetetraacetate and 385 μl of precipitation buffer are added to 50 μl of lysed erythrocytes. The mixture thus obtained is incubated for fifteen minutes on ice followed by centrifugation at 4000 rpm for ten minutes. After centrifugation, 300 μl of the supernatant is withdrawn and 750 μl of sodium diphosphate and 100 μl of 5,5-dithiobis-6,2-nitrobenzoic acid are added. The mixture thus obtained is incubated for ten minutes, after which the absorbance of the sample is measured at a wavelength of 412 Nm. The procedure for preparing the blind probe is the same as for the samples, with the same volume of distilled water being used instead of erythrocyte lysate. To determine the concentration of GSH in the samples, a calibration curve was constructed using four standards with known concentrations of glutathione [5].

Definition of catalase

Determination of antioxidant enzyme - catalase activity (CAT) was carried out according to the method of A ebi. For CAT determination, buffer, prepared lysate sample and 10 mM H_2O_2 were used. CAT activity was measured spectrophotometrically at a wavelength of 360 nm and expressed as U/mL/Hb of hemolysate [5].

Determination of superoxide dismutase

Determination of antioxidant enzyme - superoxide dismutase (SOD) activity was assessed using the epinephrine method according to Beutler. A sample of heart tissue homogenate was first mixed with carbonate buffer and then epinephrine was added to the mixture. SOD activity was measured at a wavelength of 470 nm and expressed as U/mL/Hb of hemolysate [5].

2.2. Monitoring of cardiac function *ex vivo*

On the day after completion of the 2-week treatment procedure after short-term anesthesia induced by ketamine/xylazine rats were killed by decapitation. The thoraxes were then opened by median thoracotomy and placed in saline cooled to +4º C after heart extraction. Then cannulas from Langendorff apparatus were attached to the hearts to ensure continuous coronary and retrograde perfusion. For continuous coronary perfusion, a pressure equal to 70 cm H_2O was generated. For retrograde perfusion, Krebs-Henseleit buffer with pH 7.4, temperature 37°C, and a balance of 95% O_2 and 5% CO_2 was used. Next, a sensor was placed in the left ventricle to continuously record heart rate (HR), systolic left ventricular pressure (SLVP), diastolic left ventricular pressure (DLVP), maximum derivative of pressure over time (dp/dt max), and minimum derivative of pressure over time (dp/dt min) 24h per day [2]. Coronary blood flow was measured by flowmetry. Thirty minutes after the start of cardiac perfusion, the hearts were stabilized. After cardiac stabilization, each group was subjected to 20-min global ischemia (in which blood flow was interrupted) followed by 30-min reperfusion. All cardiodynamic parameters and CF were monitored at 5-minute intervals during the 30-minute reperfusion phase [5, 50, 71].

2.3. Statistical analysis

The results are presented in the table and graph. Methods of descriptive and analytical statistics were used depending on the distribution of data and Student's T-criterion or nonparametric criterion for related samples was applied, and in case of rank comparisons - analysis of variance and posterior criterion.

Statistical processing of the obtained results was performed using the statistical package IBMSPSS version 26.0.

In order to assess the normality of distribution, Kruskal Wallis and Tukey post hoctest tests were used to compare percentage changes between groups [5]. Differences were considered significant at $p < 0.05$.

CHAPTER 3. RESULTS OF THE STUDY

3.1. Blood glucose level in experimental animals under modeling of type 1 diabetes mellitus treated with insulin, hyperbaric oxygenation, and their combination

When modeling type 1 DM in experimental animals, marked hyperglycemia up to 25.6 nmol/mL was found (Figure 3).

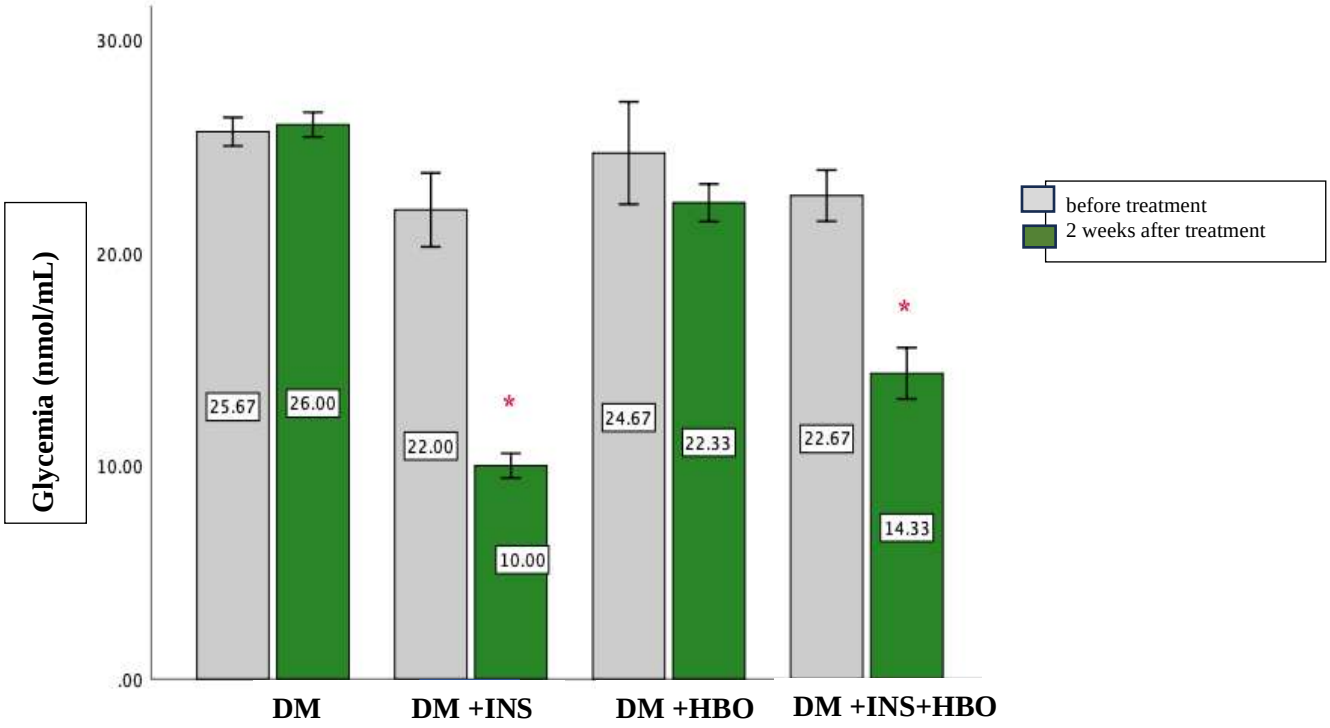

Figure 3 - Effect of insulin, HBO, and combination of insulin and HBO on glycemic levels in DM rats before and after 2-week treatment. Data are presented as mean ± standard deviation. ∗- $p < 0.05$ before and after 2-week treatment.

Treatment with insulin for two weeks significantly reduced blood glucose levels (2.2-fold $p<0.05$). HBO application reduced blood glucose levels 1.1-fold, but $p>0.05$. Thus, even HBO alone resulted in a small decrease in glycemia, but without statistical significance. The combined use of insulin and HBO reduced blood glucose 1.6-fold ($p <0.05$). Thus, insulin does not induce normoglycemia, but significantly reduces hyperglycemia in the treatment groups (DM+INS and DM+INS+HBO).

3.2. Determination of oxidative stress marker levels

Markers of oxidative stress (O_{2}^{-}, H_2O_2, TBARS and NO_{2}^{-}) were determined in coronary venous outflow tract from DM rats collected at the time of stabilization (S), then in the first minute of reperfusion after ischemia (R) [12], and then every 5 minutes during thirty minutes of reperfusion (Figure 3). These rats received insulin, HBO, and their combination.

3.2.1. Determination of superoxide *anion radical* level

The level of superoxide *anion-radical* (O_{2}^{-}) at the 1st minute of reperfusion compared to the moment of stabilization increased the most in rats with DM (3.0 times, $p < 0.05$), as well as in rats with DM receiving insulin (1.6 times, $p < 0.05$). The same index in the group receiving HBO increased 1.4-fold ($p < 0.05$) and those receiving a combination of insulin and HBO 0.9-fold ($p < 0.05$). Thus, at the 1st minute of reperfusion the level of superoxide anion radical in rats with DM, receiving insulin was 1.3 times higher ($p < 0.05$), there is also a 1.5-fold increase in the studied index ($p < 0.05$) than in rats with DM and subjected to HBO and HBO with insulin, respectively. The indicated changes were also found at the 30th minute of reperfusion process. In rats with DM without insulin correction and DM treated with insulin, the level of the studied index was 1.6 / 1.4 times higher. ($p < 0.05$ and $p < 0.05$, respectively).

Thus, the level of superoxide anion-radicals was the lowest in the group receiving the combination of HBO with insulin at almost all measurement points, both during stabilization and during reperfusion after ischemia. The greatest jump in superoxide anion radical levels was recorded at the 1st and 30th minutes of reperfusion in the DM group and the group receiving insulin. Combined treatment with insulin and HBO significantly suppressed superoxide anion radical production in the coronary venous outflow tract in DM rats, especially in the first minutes of reperfusion and to a lesser extent at the 30th minute of reperfusion after 20-minute ischemia (Figure 4).

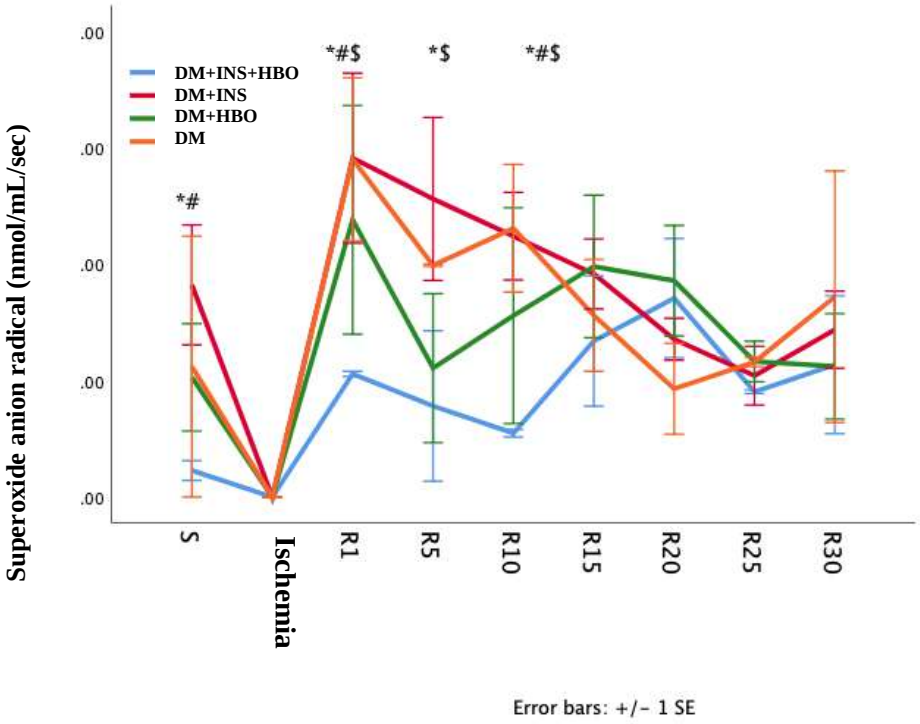

Figure 4 - Superoxide anion radical levels in DM rats (control) and in DM rats treated with insulin, HBO and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in superoxide anion radical levels. ∗p<0.05 DM group versus DM+INS group; # p<0.05 DM group versus DM+HBO group; $ p<0.05 DM group versus DM+INS+HBO group.

3.2.2. Determination of hydrogen peroxide level

In rats with DM the level of hydrogen peroxide (H_2O_2) was the highest during all 30 minutes of reperfusion. At the 1st minute of reperfusion compared to the moment of stabilization this index increased by 1.9 times (p <0.05). Treatment with insulin and HBO resulted in a slight decrease in hydrogen peroxide levels compared to the DM group, but without statistical significance. In DM rats treated with insulin or HBO, this index increased 1.3-fold (p>0.05) and 1.2-fold (p>0.05) at 1 minute of reperfusion compared to the time of stabilization, respectively. Simultaneously, rats with DM receiving the combination of insulin and HBO had a greater decrease in the level of hydrogen peroxide in the coronary venous outflow tract of rats during all 30 minutes of reperfusion (Figure 5).

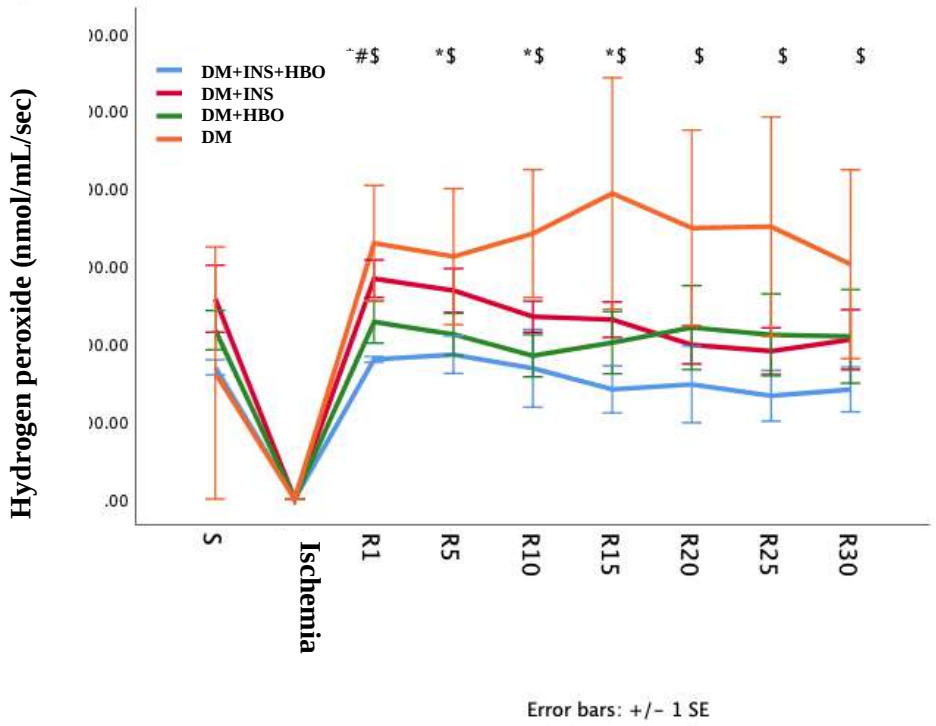

Figure 5 - Hydrogen peroxide levels in DM rats (control) and in DM rats treated with insulin, HBO, and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in hydrogen peroxide levels. ∗p <0.05 DM group versus DM+INS group; # p <0.05 DM group versus DM+HBO group; $ p <0.05 DM group versus DM+INS+HBO group.

Thus, at the 1st minute of reperfusion the level of hydrogen peroxide in rats with DM was 1.2 times higher than in rats with DM receiving insulin, 1.4 times higher ($p>0.05$) than in rats with DM receiving HBO ($p<0.05$) and 1.9 times higher than in rats with DM receiving a combination of HBO and insulin ($p<0.05$). This pattern was also registered at the 30th minute of reperfusion. In rats with DM the level of hydrogen peroxide was 1.7 times higher than in rats with DM receiving insulin and HBO (both $p<0.05$) and 2.7 times higher than in rats with DM receiving a combination of HBO and insulin ($p<0.05$)

Thus, at 15 minutes of experimental reperfusion of the myocardium of the heart of experimental animals, the level of hydrogen peroxide was the highest exactly in the DM group at all measurement points. Application of a combination of insulin and oxygenation decreased the production of H_2O_2. The most pronounced decrease was found at the thirtieth minute of reperfusion recovery processes in LV myocardium of experimental animals (male rats) (Figure 5).

3.2.3. Determination of lipid peroxidation index

In experimental animals with modeling of type 1 diabetes, the lipid peroxidation index **(**LPI) was maximal in the thirtieth minute of the reperfusion process in the heart. When studying the studied index at the first minute of reperfusion in comparison with the moment of stabilization, this index increased 3.4 times ($p < 0.05$). Treatment with insulin, HBO, and their combination, resulted in a significant decrease of LPI during reperfusion. At the first minute of the experiment (myocardial reperfusion), animals with type 1 diabetes model treated subcutaneously with insulin had an approximately 1.22-fold increase in LPI ($p > 0.05$). It was also recorded during the experiment that in animals with type 1 diabetes receiving oxygenation and the combined combination (INS+HBO), in the first minute of reperfusion compared to stabilization, LPI increased 1.1-fold and 1.1-fold, respectively, $p > 0.05$.

Drawing conclusions, when conducting the experiment with animals with experimental type 1 diabetes it was found that in the group of DM and animals with type 1 diabetes, receiving subcutaneously insulin, the studied LPI increased approximately 1.5 times, as opposed to rats with DM, with a course of oxygenation $p<0.05$, and 2.2 times, than in experimental animals with type 1 diabetes, receiving a combination of HBO and hormone ($p<0.05$). The indicated processes were reproducible and at the thirtieth minute of the experiment (Figure 6).

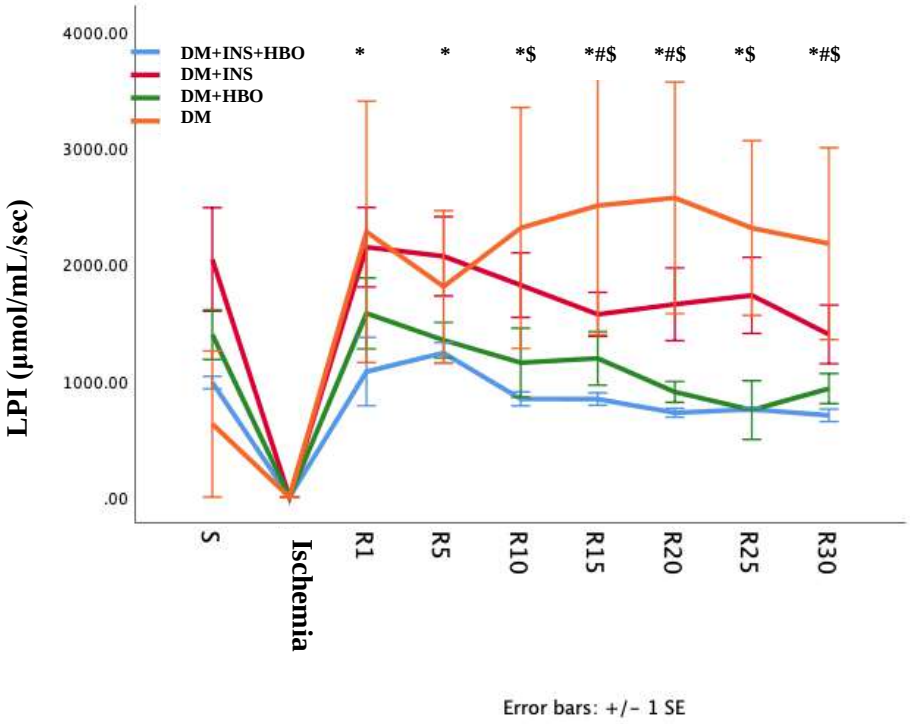

Figure 6 - LPI levels in DM rats (control) and in DM rats treated with insulin, HBO, and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in LPI levels. ∗p<0.05 DM group versus DM+INS group; # p<0.05 DM group versus DM+HBO group; $ p<0.05 DM group versus DM+INS+HBO group.

When analyzing the experimental data, we found that the studied index of lipid peroxidation processes activity was the highest in the group of type 1 diabetes, with maximum growth at the twentieth minute of reperfusion process. In the course of the study, it was revealed that the maximum low values were determined in the groups of type 1 diabetes and HBO, type 1 diabetes in experimental animals with insulin and oxygenation during the last 20 minutes of reperfusion. Combined treatment with insulin and HBO and HBO alone significantly suppressed lipid free radical production in the coronary venous outflow tract in rats with DM, at almost all measurement points, both during stabilization and reperfusion. The reduction was particularly pronounced at 30 minutes of reperfusion after 20-minute ischemia (Figure 6).

3.2.4. Determination of nitric oxide levels

In modeling of type 1 diabetes in animals, the level of the indicator determining the value of (NO_2^-) was maximal during all 30 minutes of reperfusion process. At the first minute of reperfusion compared to the moment of stabilization the studied index increased approximately 2.4 times, $p < 0.05$. HBO application significantly decreased (NO_2^-) during reperfusion exposure with myocardium of the left ventricle. In rats with DM, receiving insulin at the first min of reperfusion process in comparison with the moment of stabilization the mentioned studied index increased approximately 1.3 times, $p > 0.05$. In experimental animals with type 1 diabetes, receiving oxygenation and hormone(insulin) + HBO at the first min. of reperfusion, compared to the time of stabilization, (NO_2^-) increased 1.1 and 1.1-fold, respectively, $p > 0.05$.

Nitric oxide levels were 1.2 times higher in the DM group than in the DM+INS group ($p > 0.05$) 2.2 times higher than in the DM+HBO group ($p < 0.05$), and 2.2 times higher than in the DM+INS+HBO group ($p < 0.05$). This pattern was also registered at the 30th minute of reperfusion. Nitric oxide levels were 1.1-fold higher in DM rats than in DM rats receiving insulin ($p > 0.05$), 2.1-fold higher than in DM rats receiving HBO ($p < 0.05$), and 2.2-fold higher than in DM rats receiving the combination of HBO and insulin ($p < 0.05$) (Figure 7).

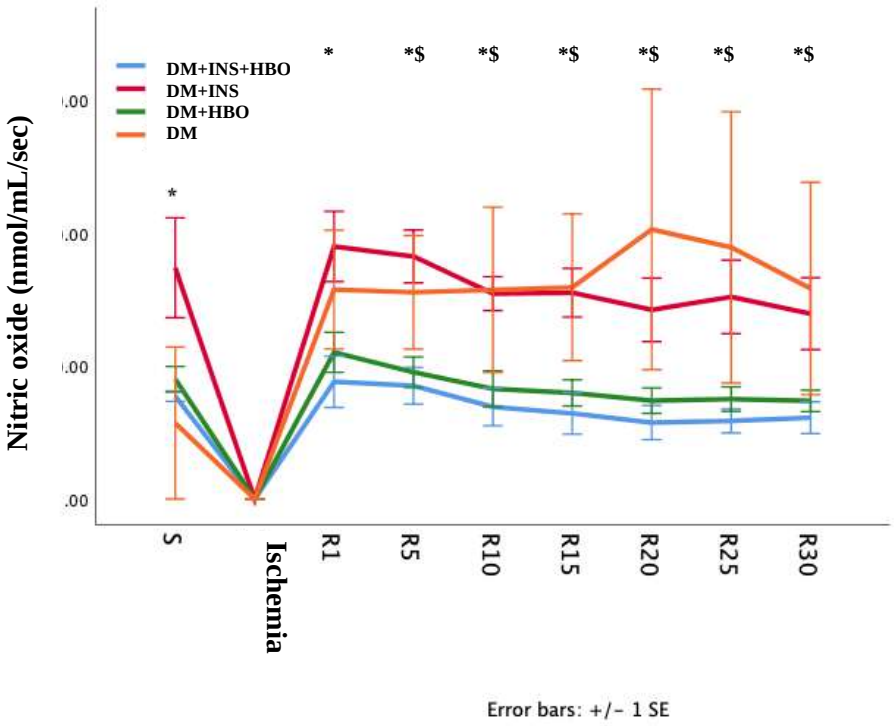

Figure 7 - Nitric oxide levels in DM rats (control) and in DM rats treated with insulin, HBO and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in nitric oxide levels. ∗p<0.05 DM group versus DM+INS group; # p<0.05 DM group versus DM+HBO group; $ p<0.05 DM group versus DM+INS+HBO group.

Thus, nitric oxide levels were highest in the DM group at all measurement points, with the largest jump at 20 minutes of reperfusion. Insulin alone did not significantly reduce nitric oxide production. Experimental data indicate that the lowest values were recorded in the DM+HBO and DM+INS+HBO groups during the last 20 minutes of reperfusion. Combined treatment with insulin and HBO and HBO alone significantly suppressed nitric oxide production in the coronary venous outflow tract in DM rats, at almost all measurement points, both during stabilization and reperfusion. The decrease was particularly pronounced at 30 minutes of reperfusion after 20-minute ischemia (Figure 7).

Table 1 presents the statistical results showing a statistically significant difference for almost all markers.

Table 1 - Results of statistical analysis obtained by comparing the values of oxidative stress markers using ANOVA analysis

Indicator	**Group**	**P**	**Group**	**P**	**Group**	**P**
ABOUT -2	DM	***0.017***	DM	0.141	DM+HBO	0.653
	DM+INS	***0.001***	DM+HBO	0.076	DM+INS+ HBO	0.639
H_2 O_2	DM	***0.032***	DM	0.124	DM+HBO	***0.026***
	DM+INS	***0.009***	DM+HBO	0.083	DM+INS+ HBO	0.063
IPOL	DM	***0.002***	DM	0.186	DM+HBO	***0.005***
	DM+INS	***0.000***	DM+HBO	0.132	DM+INS+ HBO	***0.026***
NO-	DM	***0.000***	DM	0.254	DM+HBO	***0.003***
	DM+INS	***0.000***	DM+HBO	0.225	DM+INS+ HBO	***0.025***
O -2	DM	***0.044***	DM+INS	0.629	DM+INS	0.247
	DM+INS+ HBO	***0.046***	DM+INS+ HBO	0.596	DM+HBO	0.242
H_2 O_2	DM	***0.006***	DM+INS	0.057	DM+INS	0.422
	DM+INS+ HBO	***0.007***	DM+INS+ HBO	0.134	DM+HBO	0.416
IPOL	DM	***0.004***	DM+INS	0.442	DM+INS	***0.004***
	DM+INS+ HBO	***0.005***	DM+INS+ HBO	0.506	DM+HBO	***0.002***
NO-	DM	***0.006***	DM+INS	0.818	DM+INS	***0.000***
	DM+INS+ HBO	***0.008***	DM+INS+ HBO	0.833	DM+HBO	***0.000***

Note: bold font indicates reliable difference of markers when comparing separate groups.

3.3. STUDY OF PECULIARITIES OF CORONARY CIRCULATION AND CARDIODYNAMIC PARAMETERS OF THE HEART IN EXPERIMENTAL ANIMALS

In rats with DM treated with insulin, HBO, or their combination after 20-minute ischemia followed by 30-minute reperfusion cardiodynamic parameters and coronary circulation parameters were investigated.

3.3.1. Determination of the maximum rate of pressure rise in the left ventricle in male rats in the experiment

The value of the maximum rate of left ventricular pressure rise (maximum derivative of pressure over time, dp/dt max) was the lowest in the DM group at all measurement points, both at stabilization and at reperfusion after ischemia. In DM rats, the magnitude of dp/dt max increased 1.2-fold at 1 minute of reperfusion compared with the time of stabilization ($p>0.05$). Treatment with insulin alone or in combination with HBO, as well as HBO alone caused an increase in the values of dp/dt max at almost all measurement points compared to the DM group. In DM rats receiving insulin, HBO, and combination of insulin and HBO at the 1st minute of reperfusion in comparison with the moment of stabilization this index increased 1.7 times ($p<0.05$), 1.4 times ($p<0.05$) and 1.8 times ($p<0.05$), respectively. Thus, at the 1st minute of reperfusion, the magnitude of dp/dt max in rats with DM receiving insulin, HBO, and the combination of insulin and HBO at the 1st minute of reperfusion was 1.8-fold ($p<0.05$), 1.4-fold ($p<0.05$) and 1.9-fold ($p<0.05$) greater than in rats with DM. The greatest jump in the value of dp/dt max was registered at the 15th minute of reperfusion in DM+INS+HBO group in comparison with all other groups. This pattern was also registered at the 30th minute of reperfusion. In laboratory animals - rats with type 1 experimental diabetes, receiving hormonal therapy, HBO, and combination of INS+HBO at the thirtieth minute of reperfusion the value of dp/dt max increased 3.0 times, $p<0.05$, 2.8 times ($p<0.05$) and 1.5 times ($p<0.05$) than in rats with DM (Figure 8).

Thus, insulin, HBO, and the combination of insulin and HBO resulted in an increase in dp/dt max in all determinations, both during stabilization and reperfusion (Figure 8).

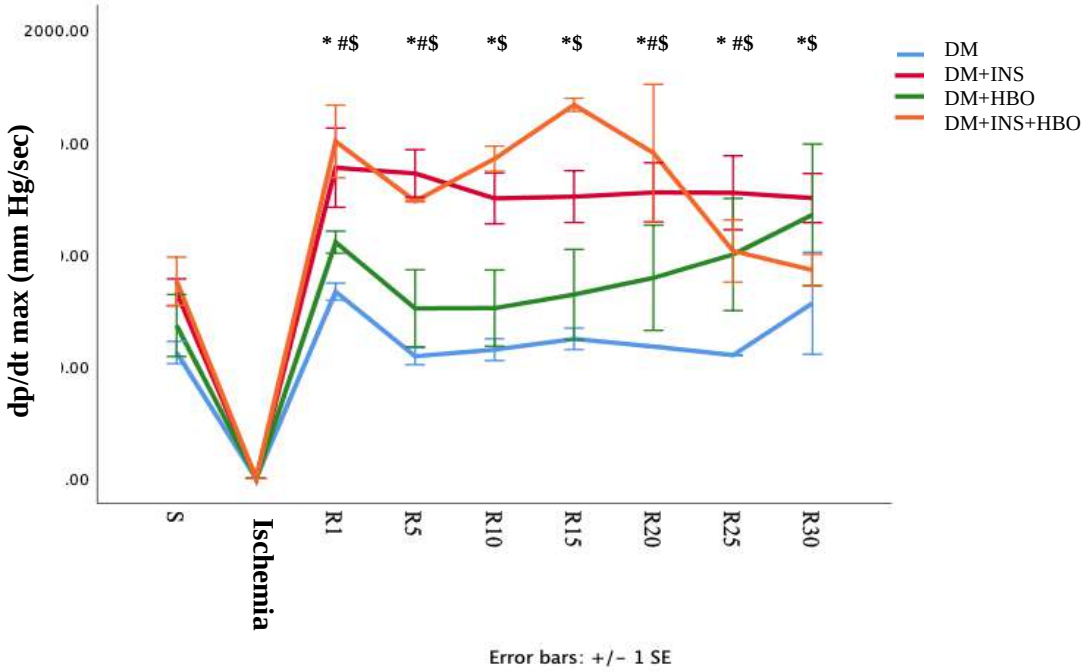

Figure 8 - Magnitude of the maximum rate of left ventricular pressure rise (dp/dt max) in DM rats (control) and in DM rats treated with insulin, HBO, and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in the magnitude of dp/dt max. ∗p<0.05 DM group versus DM+INS group; # p<0.05 DM group versus DM+HBO group; $ p<0.05 DM group versus DM+INS+HBO group.

3.3.2. Determination of the minimum rate of pressure rise in the left ventricle (dp/dt min) in experimental animals

The value of the minimum rate of pressure rise in the left ventricle (minimum derivative of pressure over time, dp/dt min) was the highest in the DM+INS+HBO group and statistically significantly higher compared to all other groups both at the time of stabilization and during reperfusion. In DM rats, the value of dp/dt min at the 1st minute of reperfusion compared to the time of stabilization was practically unchanged (p>0.05). Treatment with insulin alone or in combination with HBO, as well as HBO

alone caused an increase in the values of the value of dp/dt min at almost all measurement points compared to the DM group. In rats with DM treated with insulin, HBO, and combination of insulin and HBO at the first minute of reperfusion process in comparison with the point of stabilization the mentioned investigated index increased 1.6 times ($p<0.05$), 1.7 times ($p<0.05$) and approximately 1.4 times ($p<0.05$), respectively.

The data of experiments testify that, at the first minute of reperfusion process the value of dp/dt min in rats with experimental diabetes receiving insulin, oxygenation, and combination of insulin hormone and HBO at the first minute of reperfusion process was higher by 1.9 times ($p<0.05$), 1.7 times ($p<0.05$) and approximately 4.5 times ($p<0.05$), respectively, than in animals with experimental diabetes.

The maximum increase of dp/dt min value was found from the fifteenth to the twenty-fifth minute of the reperfusion process in the DM+INS+HBO group in comparison with all other studied groups of animals. The indicated positions maintained their values at the thirtieth minute of the reperfusion process as well. In rats with DM, receiving insulin, HBO, and combination of insulin and HBO at the 30th minute of reperfusion the value of dp/dt min was 1.2 times ($p<0.05$), 1.3 times ($p<0.05$) and 2.5 times ($p<0.05$) greater than in rats with DM (Figure 9).

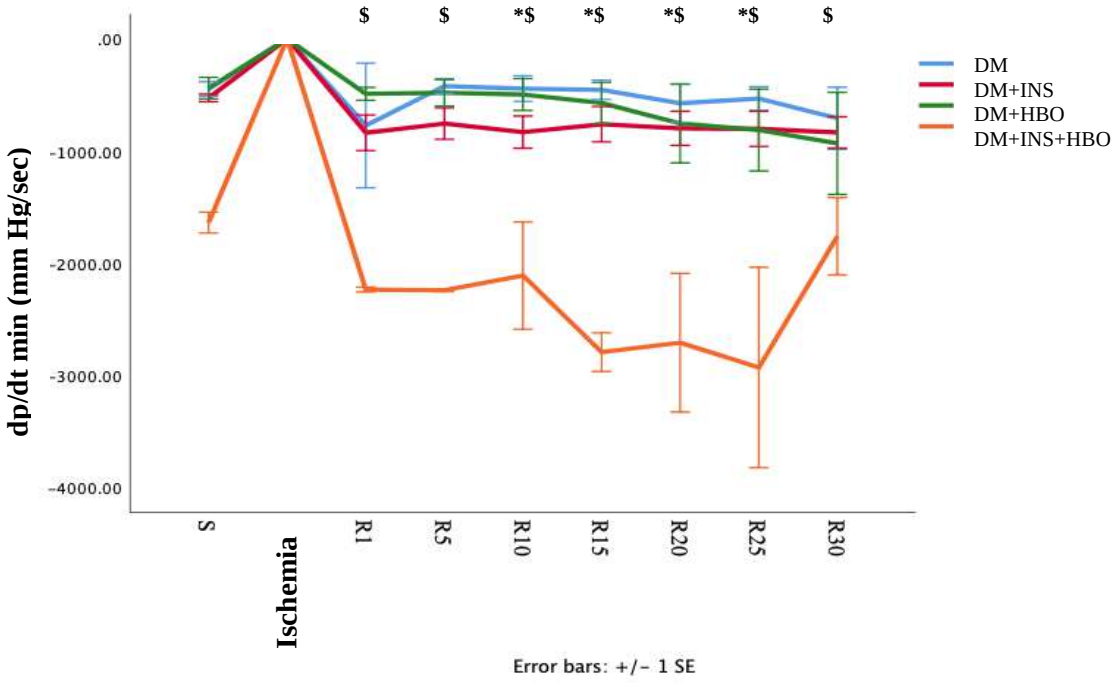

Figure 9 - Magnitude of the minimum rate of left ventricular pressure rise (dp/dt min) in DM rats (control) and in DM rats treated with insulin, HBO, and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in the magnitude of dp/dt min. ∗p<0.05 DM group vs. DM+INS group; # p<0.05 DM group vs. DM+HBO group; $ p<0.05 DM group vs. DM+INS+HBO group.

Thus, insulin, HBO, and the combination of insulin and HBO resulted in an increase in dp/dt min in all measurements, both during stabilization and reperfusion experiments (Figure 9).

3.3.3. Determination of systolic left ventricular pressure in experimental animals under modeling of diabetes mellitus and different variants of glycemia correction

The value of systolic left ventricular pressure (SLVP) was highest in the group receiving insulin alone and in combination with HBO and statistically significantly higher compared with the values in the group of experimental animals in the model of type 1 diabetes at the time of stabilization, and at reperfusion (Figure 10).

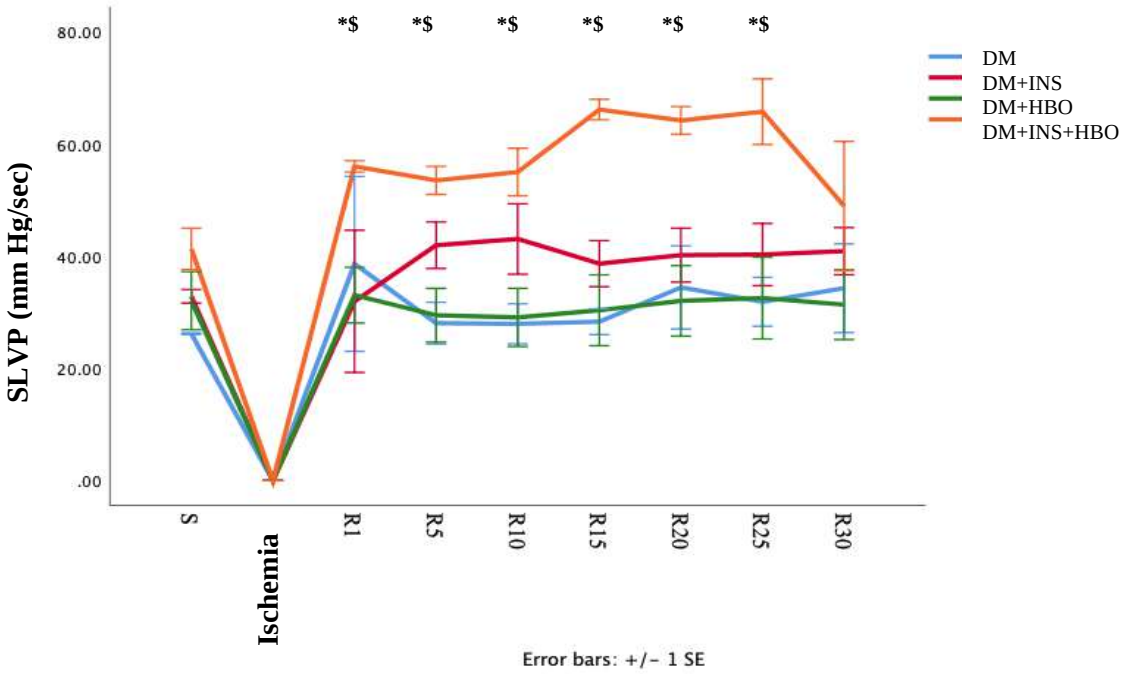

Figure 10 - Systolic left ventricular pressure in DM rats (control) and in DM rats treated with insulin, HBO, and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in systolic left ventricular pressure values. ∗p<0.05 DM group versus DM+INS group; # p<0.05 DM group versus DM+HBO group; $ p<0.05 DM group versus DM+INS+HBO group.

In laboratory animals with type 1 diabetes (experimental modeling), with DM, receiving insulin and oxygenation separately the index of SLVP at the first minute of reperfusion compared to the time of stabilization practically did not change (all p>0.05). In rats with DM receiving a combination of insulin and HBO, at the first minute of reperfusion compared to the time of stabilization, the indicated value increased approximately 1.6-fold, p<0.05. Drawing conclusions, it can be noted that during the study, at the first minute of the reperfusion process, the index of SLVP in experimental animals with type 1 diabetes, receiving a combination of subcutaneous insulin and HBO, at the first minute of the reperfusion process was recorded greater by about 1.5-fold (p<0.05), 1.7-fold (p<0.05) and 1.7-fold (p<0.05), respectively, than in rats with DM and with DM treated with insulin and HBO alone. Treatment with insulin alone or in combination with HBO induced an increase in the values of SLVP magnitude at almost all measurement points compared to the DM and DM group treated with HBO.

The largest jump of the SLVP value was registered from the 15th to the 25th minute of reperfusion in the DM+INS+HBO group and from the 5th to the 10th minute in the DM+INS group in comparison with all other groups. This pattern was also registered at the 30th minute of reperfusion. In DM rats receiving insulin and the combination of insulin and HBO, at the 30th minute of reperfusion, the magnitude of SLVP was 1.3-fold ($p<0.05$) and 1.7-fold ($p<0,05$), respectively, than in DM rats and 1.4-fold ($p<0.05$) and 1.8-fold ($p<0.05$), respectively, than in DM rats receiving HBO alone (Figure 10).

Thus, HBO alone had no significant effect on the values of SLVP. Insulin administration and combination of insulin and HBO led to an increase in SLVP at all measurement points, both during stabilization and reperfusion (Figure 10).

3.3.4. Determination of diastolic left ventricular pressure in experimental animals under modeling of diabetes mellitus and different variants of glycemia correction

Combined therapy with insulin and HBO resulted in the most significant increase in diastolic left ventricular pressure (DLVP) values compared to all other groups. In all groups, the value of DLVP at the 1st minute of reperfusion compared to the moment of stabilization was practically unchanged (all $p>0.05$). Thus, at the first minute of reperfusion, the value of DLVP in rats with DM receiving the combination of insulin and oxygenation was increased 1.5-fold than in laboratory animals with type 1 diabetes and diabetes treated with hypoglycemic therapy and HBO separately, all $p<0.05$. Administration of insulin subcutaneously in monotherapy or with HBO resulted in an increase in DLVP in almost all measurements compared with the experimental diabetes and type 1 diabetes groups with HBO (Figure 11).

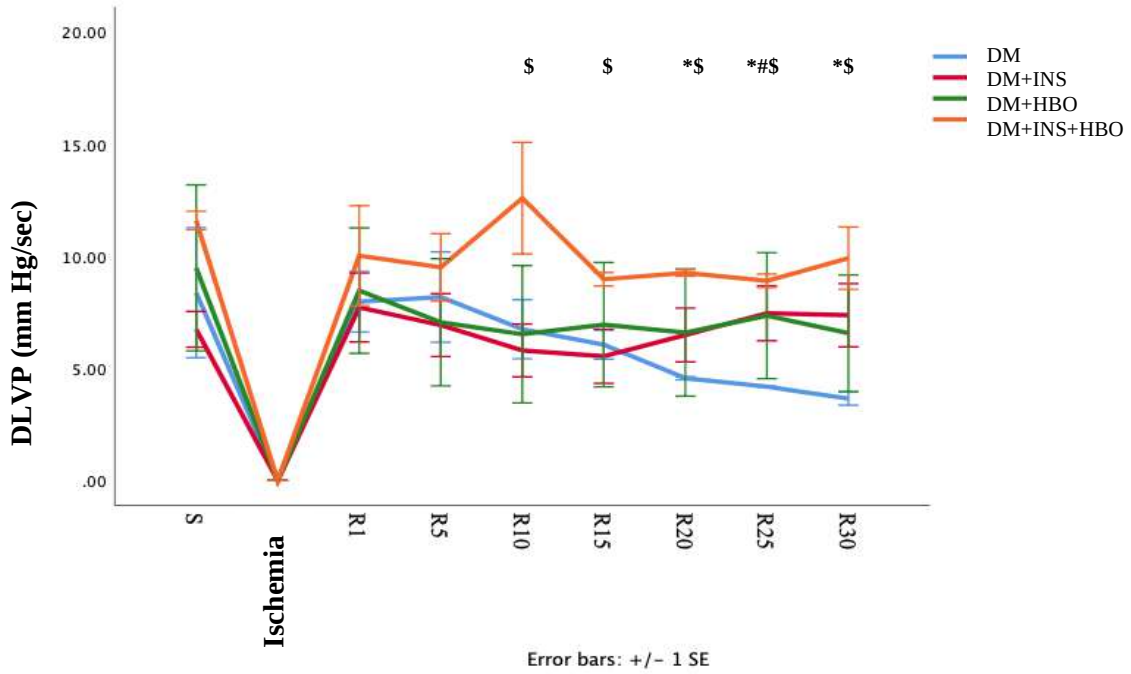

Figure 11 - Diastolic left ventricular pressure in DM rats (control) and in DM rats treated with insulin, HBO, and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in diastolic left ventricular pressure (DLVP) values. ∗p<0.05 DM group versus DM+INS group; # p<0.05 DM group versus DM+HBO group; $ p<0.05 DM group versus DM+INS+HBO group.

The maximum increase in the DLVP index was found from the tenth to the thirtieth minute of reperfusion in the group of DM+INS+HBO and from the 25th to the 30th minute in the group of experimental type 1 diabetes and INS compared to all other groups [4]. At the same time, HBO independently led to a significant increase in the values of DLVP in the last 10 minutes of reperfusion compared to the DM group. This pattern was also registered at the 30th minute of reperfusion. In rats with DM treated with insulin, HBO and a combination of insulin and HBO, at the 30th minute of reperfusion the values of DLVP were 1.9-fold ($p<0.05$), 1.8-fold ($p<0.05$) and 2.5-fold ($p<0.05$) higher, respectively, than in rats with DM (Figure 11).

Thus, administration of insulin, HBO, and a combination of insulin and HBO resulted in an increase in DLVP at all measurement points, both during stabilization and reperfusion (Figure 11).

3.3.5. Determination of heart rate in experimental animals with diabetes mellitus and different types of glycemia correction

Heart rate (HR) was the highest in the group receiving insulin alone and in combination with HBO and statistically significantly higher compared to the values in the DM group at the time of stabilization as well as during reperfusion. In all groups, HR at the 1st minute of reperfusion compared to the moment of stabilization was practically unchanged (all $p>0.05$). Treatment with insulin alone or in combination with HBO caused an increase in HR at almost all measurement points compared to the DM and DM group receiving HBO. The highest HR spike was registered from the 25th to the 30th minute of reperfusion in the DM+INS+HBO group and at the 30th minute in the DM+INS group compared to all other groups. This pattern was also registered at the 30th minute of reperfusion. In DM rats treated with insulin and combination of insulin and HBO, at the 30th minute of reperfusion HR was 1.5 times higher ($p<0.05$) than in DM rats and in DM rats treated with HBO only (Figure 12).

Thus, administration of HBO alone had no significant effect on HR. Insulin administration and combination of insulin and HBO led to an increase in HR at all measurement points, both during stabilization and reperfusion (Figure 12).

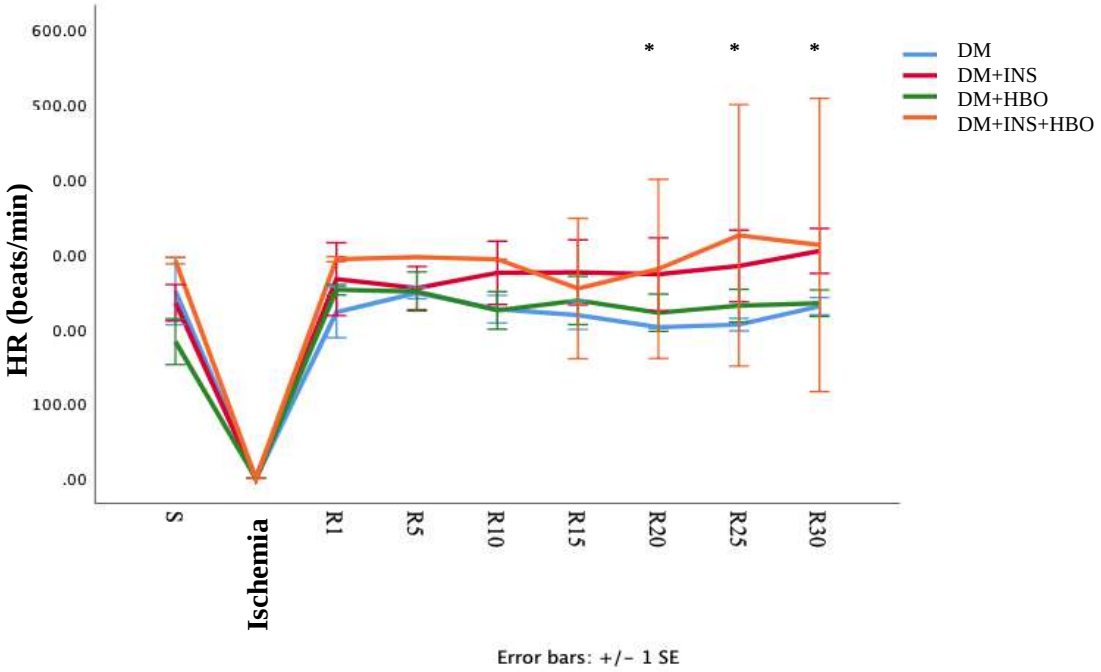

Figure 12 - Heart rate in DM rats (control) and in DM rats treated with insulin, HBO, and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in heart rate values. ∗p<0.05 DM group versus DM+INS group; # p<0.05 DM group versus DM+HBO group; $ p<0.05 DM group versus DM+INS+HBO group.

3.3.6. Determination of circulating blood volume through myocardial blood vessels in experimental animals under modeling of diabetes mellitus and different variants of glycemia correction

The DM group had the lowest blood circulation through myocardial blood vessels during all 30 minutes of reperfusion as well as during stabilization. HBO treatment resulted in a slight increase in the value of myocardial blood vessel circulation compared with the DM group, but without statistical significance, whereas insulin or its combination (DM+INS+HBO group) significantly increased myocardial blood vessel circulation during all 30 minutes of reperfusion (Figure 13). This pattern was also registered at the 30th minute of reperfusion. In rats with DM receiving insulin and the combination of insulin and HBO, at the 30th minute of reperfusion, blood circulation through myocardial blood vessels was 1.4-fold ($p<0.05$) and 2.5-fold ($p<0.05$) greater

than in rats with DM. In rats with DM receiving only HBO, this index was 1.2-fold greater than in rats with DM, but p>0.05 (Figure 13).

Thus, HBO administration alone had no significant effect on blood circulation through myocardial blood vessels. Insulin administration and combination of insulin and HBO resulted in increased blood circulation through myocardial blood vessels at all measurement points, both during stabilization and reperfusion (Figure 13).

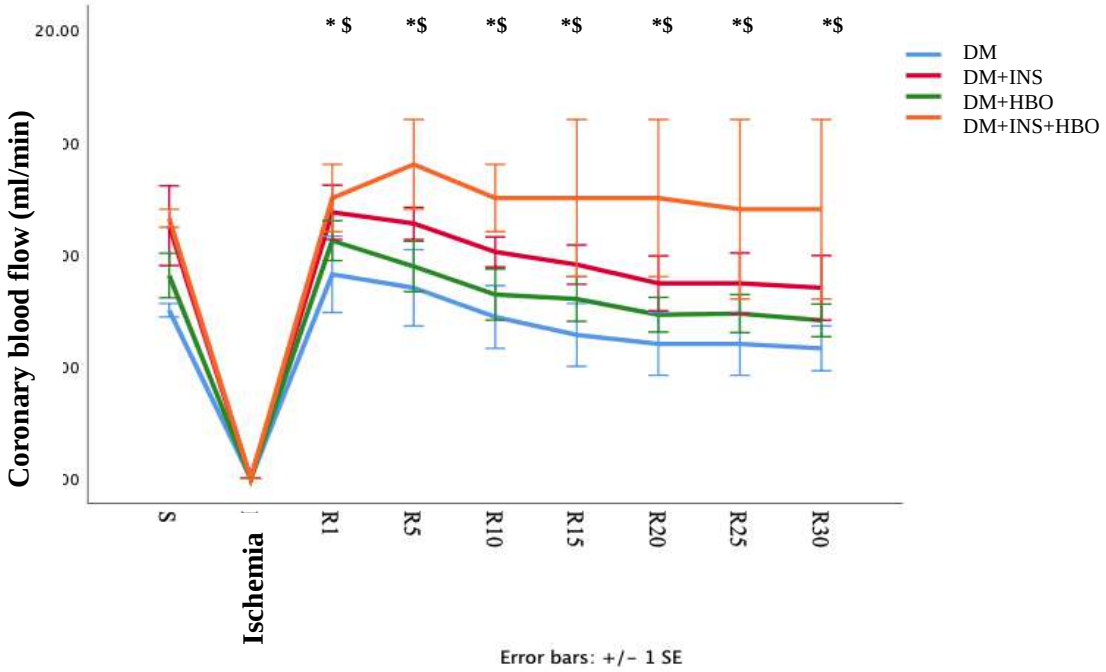

Figure 13 - Magnitude of myocardial blood vessel circulation values in DM rats (control) and in DM rats treated with insulin, HBO, and a combination of insulin and HBO. S, stabilization; I, ischemia; R, every 5 min of reperfusion. Data are presented as mean ± standard deviation. An independent t-test was used to examine differences in the magnitude of myocardial blood vessel circulation value. ∗p<0.05 DM group versus DM+INS group; # p<0.05 DM group versus DM+HBO group; $ p<0.05 DM group versus DM+INS+HBO group.

3.4. STUDY OF DYNAMICS OF CHANGES IN BIOMARKERS OF OXIDATIVE STRESS IN PERIPHERAL BLOOD IN RATS WITH DIABETES MELLITUS

One of the indicators of oxidative stress and the course of lipid peroxidation processes is superoxide anion radical. When analyzing its activity, it was found that

experimental animals with type 1 diabetes, receiving insulin, the level of this biomarker increased by 5.7% compared to the group without insulin therapy.

In the third group, where subcutaneous insulin and HBO were used, the value of this index decreased by 6.3%.

In the fourth group, when experimental diabetes was treated with hyperbaric oxygenation alone, the index of the above marker was statistically significantly decreased by about 14.7% compared to the first study group (Table 2).

When analyzing experimental data, in particular, changes in the values of the marker of lipoperoxidation process – hydrogen peroxide showed that the use of hypoglycemic therapy in the group of animals with experimental diabetes increased the level of this indicator by 26.7% compared to the group of animals without hypoglycemic therapy [5].

In the third group of experimental animals, where hypoglycemic therapy and HBO were used, the index of hydrogen peroxide increased by about 1.1%. In the fourth experimental group, when correcting type 1 diabetes in laboratory animals exclusively by hyperbaric oxygenation, the indicated index statistically significantly decreased by about 5.3% compared to the first group [5] (Table 2).

Dynamics of oxidative stress biomarker - malonic dialdehyde (by TBARS method) - showed that the use of hypoglycemic therapy in experimental animals with type 1 diabetes increased the index of the indicated marker by about 35.7% compared to the first group (without hypoglycemic insulin therapy) [5].

In the third experimental group, where hypoglycemic therapy with the hormone insulin and hyperbaric oxygen therapy was used, the index of malonic dialdehyde was reduced by about 9.4%.

In group 4, when DM was corrected by hyperbaric oxygenation alone, the level of this biomarker increased by 1.1% compared to group 1 [5] (Table 2).

Table 2 - Content of superoxide anion radical (O_{2-}), hydrogen peroxide (H_2O_2), malonic dialdehyde (MDA) and nitric oxide (NO_2) in rats with diabetes without treatment and after treatment with insulin, insulin in combination with HBO, and HBO alone [5]

Research groups	O_{2-} level nmol/mL/sec	Dynamics of O_{2-} level (%)	H_2O_2 level nmol/mL/sec	Dynamics of H_2O_2 level (%)	MDA level micro/mol/mL/sec	Dynamics of MDA level (%)	NO_2 level nmol/mL/sec	Dynamics of NO_2 level (%)
I. Untreated DM	3,82±0,38		5,38±0,43		1,82±0,17		4,91±0,50	
II. Treatment of DM+INS	4,04±0,38	+5,7	6,82±0,54	+26,7	2,47±0,25 *	+35,7	2,23±0,22 *	-54,6
III. Treatment of DM+INS+ HBO	3,58±0,38	-6,3	5,44±0,51	+1,1	1,65±0,15 #	-9,4	4,51±0,45 #	-8,2
IV. Treatment of DM+HBO	3,26±0,33 *#	-14,7	5,10±0,50* #	-5,3	1,84±0,17 #	+1,1	4,70±0,47 #	-4,3

Note: DM - diabetes mellitus, INS - insulin therapy, HBO - hyperbaric oxygenation; * - $p < 0.05$ compared to control group 1, # - $p < 0.05$ compared to insulin treatment group 2 [5].

In a series of experiments, it was found that the level of nitric oxide (NO_{2-}) in the experimental group where laboratory animals with type 1 diabetes received hypoglycemic therapy was statistically significantly reduced by about 54.6% compared to the group without hypoglycemic therapy.

In the group of experimental animals where hypoglycemic therapy in combination with hyperbaric oxygenation was used stable nitric oxide (nitrite/nitrate) decreased by 8.2% [5].

In the fourth group, HBO was used exclusively to treat type 1 experimental diabetes mellitus, and it was found that the level of stable nitric oxide indices (nitrite/nitrate) decreased by about 4.4% compared to the first group of laboratory animals [5] (Table 2).

In the study of antioxidant activity of blood in experimental animals it was found that the activity of the enzymatic link of the antioxidant system of blood, in particular SOD, showed that the use of hypoglycemic therapy in the group of animals with experimental type 1 diabetes, the activity of the above enzyme increased by 12.2% compared to the group of animals without hypoglycemic treatment [5].

In the third group, where hypoglycemic therapy combined with hyperbaric oxygen therapy was used, SOD increased by 50.2%.

In the fourth group of laboratory animals, in which only hyperbaric oxygenation therapy was used to normalize glycemia parameters, the index of antioxidant enzyme activity statistically significantly increased by about 61.4% compared to the first group of animals (Table 3).

In our study, we found that the activity of another representative of the antioxidant system of the blood - catalase, when the hormone insulin correction of glycemia level in experimental animals with type 1 diabetes, the activity of this enzyme increased by 22.2% compared to the first group without hypoglycemic treatment with insulin.

In the third group of laboratory animals, where hypoglycemic therapy with short-acting insulin and hyperoxygenation was applied, the index of catalase activity statistically significantly increased by about 108.3%.

In the fourth group of experimental animals at correction of type 1 diabetes in male rats exclusively by hyperbaric oxygenation in monotherapy mode, the index of catalase activity statistically significantly and significantly increased by 115.2% in comparison with the first group of laboratory animals [5] (Table 3).

Table 3 - Activity of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in diabetic rats without treatment and after treatment with insulin, insulin in combination with HBO, and HBO alone [5]

Research groups	SOD activity of U/mL/Hb hemolysate	SOD activity dynamics (%)	CAT activity of U/mL/Hb hemolysate	CAT activity dynamics(%)	GSH activity of U/mL/Hb hemolysate	GSH activity dynamics (%)
I. Untreated DM	19,44±1,20		2,88±0,25		16497,5±1550,0	
II. Treatment of DM+INS	21,82±2,17	+12,2	3,52±0,38	+22,2	18090,1±1700,2	+9,6
III. Treatment of DM+INS+HBO	29,20±2,53 *#	+50,2	6,00±0,30 *	+108,3	30387,7±2965,3 *#	+84,2
IV. Treatment of DM+HBO	31,34±2,0* #	+61,2	6,20±0,32 *#	+115,2	14120,9±1270,5 #	-14,4

Note: DM - diabetes mellitus, INS - insulin therapy, HBO - hyperbaric oxygenation; * - $p < 0.05$ compared to control group 1, # - $p < 0.05$ compared to insulin treatment group 2 [5].

The next stage of our study was to investigate the activity of one of the main representatives of the antioxidant system of blood - reduced glutathione (GSH). When analyzing laboratory data, we found that the use of insulin in type 1 experimental diabetes in the 2nd group of animals increased the level of activity of this enzyme by 9.6% compared to the first group without hypoglycemic therapy [5].

In the third group of laboratory animals, where a combination of insulin therapy and HBO was used, the index of glutathione activity increased statistically significantly by 84.2% [5].

In the fourth group of laboratory animals at correction of glycemia level in experimental animals with type 1 diabetes by hyperbaric oxygenation only, the index of reduced glutathione activity decreased by 14.4% in comparison with the first group of male laboratory rats [5] (Table 3).

Thus, it was found that receiving only HBO therapy in rats with DM resulted in a decrease in blood parameters that are biomarkers of oxidative stress and an increase in the activity of antioxidant enzymes, reflecting the onset of antioxidant effects.

CHAPTER 4. DISCUSSION OF THE RESULTS OBTAINED

DM is characterized by the development of acute (coma) and chronic (micro/macrovascular) complications [125, 166]. Although numerous studies have examined and compared the roles of various components that contribute to vascular complications in DM, accurately assessing their influence remains a challenge [63]. Nevertheless, several studies have shown that both microvascular and macrovascular outcomes are mainly or partially dependent on the level and constancy of hyperglycemia and its diurnal fluctuations [24]. Vascular damage caused by hyperglycemia involves at least four major pathways: increased polyol activity, which causes accumulation of sorbitol and fructose; increased formation of glycation end products; activation of protein kinase C and nuclear factor kB; and increased hexosamine levels. There are many reasons indicating that hyperglycemia causes all these metabolic disturbances through one single pathway: because the mitochondrial electron transfer chain produces too much superoxide anion radical. This theory, proposed by Brownlee [41], seems to show that oxidative stress induced by hyperglycemia plays an important role in the pathogenesis of DM. Chronic (low intensity) inflammation also has a significant impact on the development of DM. The presence of inflammation triggers the production of reactive oxygen species by tissues or immune cells in various peripheral tissues, including adipose tissue (especially on the anterior abdominal wall), atherosclerotic plaques with vascular endothelium, liver (due to triglyceride accumulation), intestine, etc., and other tissues. Active oxygen species bind to certain subunits of the insulin receptor, affecting postreceptor signaling pathways, preventing the body from properly responding to insulin levels [159].

A valid experimental model of DM1 in experimental animals (rats) is the use of STZ. To gain a full understanding of the role of HBO and glycemic control in myocardial physiology and pathophysiology, ex vivo myocardial function was monitored using a Langendorff apparatus in rats with type DM1 through the function of the left ventricle, which is morphologically and functionally the more dominant cardiac cavity. In addition, the Langendorff apparatus was infrared damaged and cardiac function was

monitored during the reperfusion period. Thus, HBO was investigated as a potential preconditioning agent. Enhancement of fibroblast replication, increased collagen production, and neovascularization of ischemic tissues are the most significant benefits of HBO in ischemia-reperfusion injury. It is proved that HBO increases the oxygen level in arterial blood. The increase of tissue-cell diffusion gradient increases oxygen supply to cells, and moderate hyperoxia enhances angiogenesis by increasing the formation of necessary collagen matrix for angiogenesis [44, 176]. To evaluate the above changes in coronary venous outflow tract of isolated rat heart, oxidative stress parameters were measured. In this model, a significant increase in the level of TBAR, which is a marker of lipid peroxidation, was recorded, indicating the existence of oxidative stress [5, 15, 46, 56]. From a series of experiments, we have shown that combined treatment with insulin and HBO leads to a significant decrease in TBAR production in coronary venous outflow tract. As a by-product of lipid peroxidation, MDA is sometimes considered as a sign of oxidative stress. The findings of some authors [5, 73] that HBO administration before ischemia improved renal blood flow by reducing radical oxygen peroxidation of membrane lipids are consistent with our results. However, there are data that contradict our findings. For example, in [165], although MDA levels did not reach a statistically significant level, they were higher in the groups receiving HBO than in the other groups. In this study, elevated MDA levels were considered as a marker of oxidative damage, which was hypothesized to be caused by increased cellular oxygen levels because of HBO administration. A balance between oxidative and antioxidant systems can be achieved by increasing antioxidant levels in the same groups, which may reduce pro-oxidative damage. It was also demonstrated that 30 min after HBO with 30% oxygen pressure at 1.3 ATM in dogs, glutathione peroxidase activity in erythrocytes was increased. Nuclear erythroid-related factor 2 (Nrf2), a redox-sensitive transcription factor, is thought to play a key role in cellular defense against oxidative stress by upregulating the transcription of antioxidant stress proteins such as glutathione peroxidase and phase II defense enzymes [1, 6, 66]. Oxidative stress-related changes in the mRNA and protein expression of antioxidant enzymes such as CAT and copper and zinc SOD were found in the liver tissues of rats

with STZ-induced DM [66]. These results support the assumption that the pathological processes leading to the realization of DM are based on an enhanced course of oxidative stress [87, 91, 148, 157]. In the current study, we have shown that 2-week treatment with insulin alone or in combination with HBO causes a decrease in the production of oxidative stress markers in rat coronary venous outflow tracts, which were collected after 20-min global ischemia (in which blood flow was interrupted), and during 30-min reperfusion [5, 15, 70, 71]. These results are consistent with a study that investigated the effects of HBO in people with DM, where it was demonstrated that HBO had no pro-oxidant effect [69]. The antioxidant enzyme system also seems to be actively involved in this process, enhancing the defense against oxidative damage [52].

Several groups of researchers [170,171,174] have demonstrated how in human endothelial cells HBO leads to an increase in the expression of antioxidant genes, which contributes to the activation of protective mechanisms against oxidative damage of ischemic tissues by reducing the production of ROS. Furthermore, it has been demonstrated that 7 daily HBO sessions can reduce the expression of hypoxia-inducible factor 1 alpha in hypoxia-induced hypoxia in ischemic rat wound tissues, consequently reducing apoptosis. In addition, these data showed that fewer neutrophils were found at the wound injury site after a course of HBO, indicating a reduction in inflammation.

A series of experiments indicate that the expression of molecular chaperones is upregulated in microvascular endothelial cells in response to HBO. Although the exact role of these chaperones in endothelial cells is still unknown, our functional studies suggest that they may help protect cells from the damaging effects of oxidative stress. Nrf-2 has been identified as a key mechanism of oxidative stress response that is controlled by HBO in terms of endothelial cell protection. More than 200 genes involved in defense and antioxidant activity are regulated by this pathway. Numerous Nrf-2 target genes can be activated upon exposure to HBO, and it is likely that these genes help protect endothelial cells from oxidative stress. Nrf-2 may control metallothioneins, which respond best to HBO. The hemoxygenase gene is one of the interesting genes regulated by Nrf-2 that is activated by HBO [21, 29, 42, 79]. Regarding DM compensation, repeated courses of HBO for a year reduce insulin

consumption, restore residual insulin secretion, and suppress the production of counterinsular hormones such as glucagon, adrenaline, and glucocorticoids. For patients with type 1 DM and patients with type 2 DM receiving insulin, it is more effective to prescribe 3 courses of HBO with an interval of 4 months than 2 courses with an interval of 6 months; however, with three-course treatment, the maximum positive effect of HBO on hormonal and metabolic status is achieved only during the 2nd course, and the 3rd course only enhances the already achieved effect [18, 19]. Combined treatment with HBO and insulin demonstrated not only a favorable effect on coronary oxidative status, but also a positive effect on cardiodynamics of the isolated rat heart. SLVF and dp/dt max are two cardiodynamic parameters that describe systolic function, whereas the parameters dp/dt min and DLVP are related to diastolic function of the heart. All four mentioned cardiodynamic parameters as well as coronary blood flow were improved in the group of rats receiving combined treatment (DM+INS+HBO), which is consistent with the results of similar studies [18, 19].

The study of such peripheral blood parameters as O_{2-}, H_2O_2, PLI and NO_{2-} , reflecting the level and dynamics of changes in the development of oxidative stress, revealed a tendency to their reduction in the groups treated with insulin, HBO and a combination of insulin and HBO compared to the group with DM [5]. The most significant reduction of oxidative stress biomarkers was recorded in the groups treated with insulin and combination of insulin and HBO. It was found that in rats with type 1 DM the isolated application of HBO leads to a decrease in blood parameters reflecting the level of oxidative stress, from which it can be concluded that this method of treatment has an antioxidant effect.

When studying the dynamics of blood enzymes involved in antioxidant defense: SOD, CAT and GSH, on the contrary, a tendency to their increase was found in the groups treated with insulin, HBO and combination of insulin and HBO compared to the group with DM. The obtained data coincide with the results of scientific studies by other authors [4, 19, 119], in which it was demonstrated how HBO enhances the expression of antioxidant genes in human endothelial cells, protecting the tissue in a hypoxic state from the harmful effects of oxidative stress. In this research work,

statistically reliable, significant increase in the considered indicators of antioxidant system was registered in the groups of DM+INS+HBO and DM+HBO, respectively. It was also found that both isolated and combined with insulin exposure to HBO causes an increase in the activity of antioxidant blood enzymes in rats with DM, which reflects its antioxidant effect [5].

Thus, the conducted studies demonstrate the positive effect of hyperbaric oxygenation as an additional component to insulin therapy in the presence of type 1 DM on the change of indicators reflecting the level of the course of reactions that are markers of oxidative stress, and the realization of antioxidant action in experimental animals.

CHAPTER 5. CONCLUSION

Population growth and scientific and technological progress, which contributes to the modification of diagnostic methods, lead to an increase in the incidence and prevalence of DM, which are on the verge of epidemic thresholds. Therefore, DM maintains its primacy among the priority problems of the public health system [7, 120]. The age of patients with DM 18 years and older in the general population as of 2014 was 8.5%. In 2019. 1.5 million deaths were directly related to diabetes. DM was also responsible for 460,000 deaths from kidney disease. Elevated blood glucose levels are the leading cause of 20% of CVD deaths for 2019. [120]. There is no doubt that adequate insulin treatment is essential for glycemic control. Glycemic control is certainly important in a condition such as DM, but it is also important to remember that insulin de facto also contributes to oxidative stress [119].

Hyperbaric oxygen treatment involves exposing the patient to barometric pressure above normal (1 ATM) while breathing 100% O_2. HBO increases the amount of dissolved oxygen in the blood, resulting in relief and elimination of clinical symptoms of conditions such as hypoxia, acute carbon monoxide poisoning, air embolism, and diabetic lower extremity lesions [64]. Even though HBO therapy is performed in the treatment of many nosologies, one should not forget about its potentially adverse effects. Scientists have been discussing for quite a long time about the influence of HBO side effects, realized in the form of strengthening the course of reactions caused by oxidative stress and manifestations of oxygen toxicity [11, 176]. On the one hand, enhancement of ROS production has positive effects: there is an increase in the synthesis of growth factors (vascular endothelial growth factor (VEGF), placental growth factor (PGF) and angiopoietins 1 and 2) and attraction of stem cells from the bone marrow responsible for neovascularization [11, 78]. On the other hand, the intensification of reactions observed in oxidative stress increases glycemia levels, which contributes to the development and progression of DM complications. And activation of lipid peroxidation in the membranes of muscle cells causes the formation of insulin resistance. It is known that DM provokes activation of processes characterizing

oxidative stress [11, 41], so prolonged exposure to HBO can potentially enhance the occurrence and development of adverse changes in organs and tissues, which leads to the progression and rapid course of the disease and the appearance of its early and late complications [11]. Despite this, to date there are no comprehensive studies that could evaluate the positive and toxic effects of HBO.

The term "ischemia-reperfusion injury" refers to the worsening of cellular damage and dysfunction after restoration of blood flow in previously ischemic tissues. ROS have been identified as primary mediators of this phenomenon. During ischemia-reperfusion injury, there is increased migration of microcirculating neutrophils between blood and endothelial cells, which induces tissue necrosis and initiates a feedback loop culminating in greater ROS production and damage [79]. However, preservation of ischemic tissues requires early restoration of blood flow. Simultaneous ischemia and reperfusion in tissues represent a paradoxical process in which reperfusion causes greater tissue damage, which is ROS-dependent. It has been suggested that tissues in a hyperoxic environment provided by HBO after ischemia-reperfusion injury would have increased production of ROS and exacerbated the extent of tissue necrosis. Unexpectedly, it has been shown how HBO leads to increased expression of antioxidant genes in human endothelial cells, which contributes to the activation of protective mechanisms against oxidative damage to ischemic tissues by reducing the production of ROS observed during the ischemia-reperfusion process [5, 170, 171, 174]. As a result, HBO seems to benefit ischemic tissue by reducing the formation of ROS and simultaneously increasing their degradation.

Thus, the aim of this study was to determine the effects of HBO on glucose homeostasis, oxidative stress parameters, and cardiac function in experimentally induced type1 DM and rats receiving and not receiving NPH insulin therapy.

DM is characterized by the development of certain microvascular problems [35]. Although numerous studies have investigated and compared the roles of various components contributing to diabetic vascular problems, the exact assessment and their contribution remains incompletely understood [132]. However, several studies have shown how microvascular outcomes are largely or partially dependent on

hyperglycemia [125]. Hyperglycemia-induced vascular damage involves at least four major pathways: increased polyol (sorbitol) activity causing accumulation of sorbitol and fructose; increased formation of glycation end products; activation of protein kinase C and nuclear factor kB; and increased flux of the hexosamine pathway. There are many reasons to believe that hyperglycemia causes all these harmful metabolic processes through one pathway: the mitochondrial electron transport chain produces too much superoxide. This theory, proposed by Brownlee [41], seems to indicate that oxidative stress induced by hyperglycemia plays an important role in the etiology of diabetic problems. On the one hand, hyperglycemia causes oxidative stress and on the other hand, insulin administration also causes oxidative stress.

In clinical practice, it has been observed that HBO can accelerate wound healing. Since wounds need oxygen for proper tissue regeneration, exposure to 100% oxygen accelerates this process. Applications in this area are quite extensive and include microbial-infected wounds (e.g., clostridial myonecrosis and Fournier's gangrene), traumatic wounds, thermal burns, skin grafts, radiation wounds, diabetic ulcers, and vascular insufficiency ulcers [119, 141].

In the field of diabetes, there is a critical complication called diabetic foot ulcer, an open ulcer on the sole of the foot affecting 15% of patients. HBO is particularly important in this injury because it is involved in many parameters of inflammation and tissue repair. For example, there is some evidence that HBO can improve the rate of wound healing by increasing NO levels and the number of endothelial progenitor cells in non-healing vasculitis, calcifying uremic arteriolopathy, lividoid vasculopathy, and gangrenous pyoderma ulcers [32, 40, 58, 78, 102, 153]. Some studies show marked angiogenesis when inflammation is reduced: angiogenic markers such as epithelial growth factor (EGF) and VEGF become active and are positively associated with an increase in the transcription factor Nrf2 [78, 88, 102, 148, 176]. In addition, anaerobic infections have lower morbidity and amputation rates [153]. Various literatures support the additional use of systemic but not local HBO for wound healing in diabetic foot ulcers [44, 142, 171]. Despite this, the results of many studies cannot be interpreted unambiguously, which makes it important to predict in advance in which group of

patients the maximum benefit of HBO application will be obtained [142]. For example, in patients with diabetic foot ulcers and peripheral arterial occlusive disease, wound healing may not improve [142]. Another recent study showed that the use of HBO may be associated with improved 6-year survival in patients with diabetic foot [142]. Further studies and larger samples are needed to determine the most appropriate candidates for HBO.

In addition, HBO can be an excellent adjuvant treatment for surgical injuries and is crucial because it can provide better results when administered earlier. When wounds fail to heal with conventional treatments, additional help can be found in HBO. Animal models have shown the importance of this procedure in wound healing by accelerating epithelialization and neovascularization [170, 171, 174, 176, 181]. Reported effects on these events are found in the upregulation of host factors such as tumor necrosis factor-α (TNF-α), matrix metallopeptidase 9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) [176, 181]. In a rabbit model of irradiated NBOT tissue, O_2 was compared to hyperbaric tissue, further demonstrating that O_2 is required at higher pressures to induce angiogenic effects [100]. Numerous *in vivo* studies have shown that hyperbaric oxygen stress modulates the rate of stem cell proliferation in small intestinal crypts and enhances angiogenesis in the chorio-allantoic membrane [153]. In a clinical study of patients with chronic non-healing wounds (more than 20 months without healing), HBO was standardized for 20 sessions (5 sessions per week). The results were increased levels of vascular endothelial growth factor and interleukin-6 and decreased levels of endothelin-1. These facts include activation of host wound healing factors, angiogenesis, and improved vascular tone [153]. Angiogenesis is enhanced by increasing NO levels during HBO and is associated with a decrease in lesion area [40, 176].

Currently, there are several studies evaluating the improvement of angiogenesis and tissue healing after HBO. However, there are still few studies in this area, and an updated metanalysis and systematic review of the available data will be published soon [162, 177]. Similar findings can be extrapolated to radiation-induced lesions. Thus, osteoradionecrosis is a common and alarming condition in cancer patients after

radiation therapy. This condition often results in jaw lesions and consists of the development of aseptic avascular necrosis, which can lead to infection, tooth loss, and even pathologic fracture of the jaw. In addition, it also often leads to ulceration and necrosis of the mucosa. HBO plays a key role in the treatment of this condition, improving tissue response to surgical wounding and even as a prophylactic approach in patients with prior head and neck irradiation undergoing tooth extraction or complete tooth extraction [103].

In the current study, we show that 2-week treatment with NPH insulin alone or in combination (which is even more pronounced) induces increased production of prooxidant markers in rat coronary venous secretions that were collected after 20 minutes of global ischemia in which blood flow is interrupted followed by 30 minutes of reperfusion. Based on the above, the data from the study by Monnier et al. (2011) do not specifically explain whether high insulin doses can be considered a factor or a reflection of oxidative stress, but it seems more likely that the degree of hyperinsulinization (high insulin doses) is responsible for the pro-oxidant effect. This view is based on the fact that there is a correlation between insulin dose and the rate of urinary excretion of isoprostane [119], which is a stable biomarker of oxidative stress [66].

Although some investigators [170, 171, 174] have shown how, in human endothelial cells, HBO leads to increased expression of antioxidant genes, contributing to the activation of protective mechanisms against oxidative damage to ischemic tissues by decreasing the production of ROS while increasing damage from their degradation, HBO also increases ROS production at the beginning of therapy**.**

Thus, our results are consistent with the above conclusions, given that rats on NPH insulin therapy and undergoing HBO had significantly higher values of all determined prooxidants compared to all other groups. Regarding compensation in DM, there is a decrease in insulin consumption, restoration of residual insulin secretion and suppression of secretion of anti-insular hormones such as glucagon, somatotropic hormone and hydrocortisone, which are achieved by repeated HBO for a year. For patients with insulin-dependent DM, 3 courses of HBO at 4-month intervals are more

effective than 2 courses at 6-month intervals. However, with the three-course modality, the maximum beneficial effect of HBO on hormonal and metabolic status is achieved only in the second course, and the third course only enhances the already achieved effect [18, 19].

Combined treatment with HBO and NPH insulin has a poor effect on coronary oxidation status but a positive effect on cardiodynamics of the isolated rat heart. SLVP and dp/dt max are two cardiodynamic parameters that describe systolic function, whereas dp/dt min and DLVP parameters refer to diastolic function of the heart. All four mentioned cardiodynamic parameters as well as CF were elevated in the group of rats with combined treatment (DM+INS+HBO), which is consistent with the results of studies [115, 168]. Some authors have shown that HBO causes a slow decrease in HR [115], but this was not observed in our study.

INFERENCES

1. A 2-week course of insulin therapy significantly reduces the level of hyperglycemia in rats with type 1 diabetes. The combination of insulin therapy with sessions of hyperbaric oxygenation causes a similar, but less pronounced effect. The smallest decrease in glucose level is observed with "isolated" therapeutic application of HBO.

2. After 2-week sessions of HBO, insulin therapy, as well as their combined application, oxidative stress characteristic of type 1 diabetes is weakened. The molecular mechanisms of this effect are based on a decrease in the activity of pro-oxidants with simultaneous activation of antioxidant system components.

3. Two-week therapeutic course of HBO reduces the activity of pro-oxidants measured in the coronary venous outflow tract of isolated rat heart under its post-ischemic reperfusion conditions. The similar in expression decrease of oxidative stress markers is observed after a course of insulin therapy.

4. Combination of HBO sessions with insulin therapy causes the greatest reduction of oxidative stress indicators (registered on the isolated heart preparation) in comparison with each of them separately.

5. The best indices of the pumping function of the rat heart under conditions of its postischemic reperfusion were observed after the combination of insulin therapy and HBO courses. The worst parameters were observed in animals that did not receive treatment. Separate application of both HBO and insulin therapy caused intermediate effects.

6. The molecular mechanisms of cardiac pumping function improvement after sessions of HBO, insulin therapy, and their combined effect are based on the attenuation of oxidative stress characteristic of type 1 DM.

ABBREVIATIONS AND SYMBOLS

ACE – angiotensin-converting enzyme

ATM - absolute atmosphere

ATP- adenosine triphosphate

CAT – catalase

CHF - chronic heart failure

CVD - cardiovascular disease

DLVP – diastolic left ventricular pressure

DM – diabetes mellitus

DM1 – type 1 diabetes mellitus

DM2 – type 2 diabetes mellitus

dp/dt max - maximum derivative of pressure over time

dp/dt min - minimum derivative of pressure over time

GLP-1 – glucagon-like peptide-1

GSH – reduced glutathione

HbA1c – glycated hemoglobin or glycohemoglobin (A1c)

HBO - hyperbaric oxygenation

H_2O_2 – hydrogen peroxide

HRPO – Horser Radish PerOxidase

HR – heart rate

INS - insulin therapy

LPI – lipid peroxidation index

MDA – malonyl dialdehyde

NO – nitrogen monoxide

NO_{2-} - nitrite

NPH – neutral protamine Hagedorn insulin or isophane insulin

Nrf-2 is a redox-sensitive transcription factor

O_{2-} – superoxide anion radical

PQTI – prolonged QTc interval

RAAS – renin-angiotensin-aldosterone system

ROS – reactive oxygen species

SLVP – systolic left ventricular pressure

SOD – superoxide dismutase

STZ – streptozotocin

TBARS – Thiobarbituric Acid Reactive Substances

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