ORIGINAL ARTICLE

CONTENTS

# The study of the hypoxia markers against the background of prolonged hyperglycemia in experimental animals

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## ABSTRACT

**Aim:** The aim of article is to investigate the energetic functions of retinal mitochondria under conditions of chronic hyperglycemia to understand the pathogenic mechanisms involved in the development of this diabetic complication.

**Materials and Methods:** The experimental study was carried out on non-linear rats which were divided into 2 groups: intact animals and rats with diabetic retinopathy. We investigated the level of lactate, pyruvate and adenine nucleotides.

**Results:** The increase in lactate and pyruvate levels may indicate a reduction in the energy potential of the retina under conditions of experimental diabetic retinopathy, while the elevation of adenine nucleotides activity suggests the activation of energy processes and the development of adaptation to hypoxia in retinal cells.

**Conclusions:** The analysis of lactate and pyruvate levels, as well as adenine nucleotides, may serve as prognostic markers in predicting the severity of diabetic retinopathy in the context of prolonged hyperglycemia.

**KEY WORDS:** diabetic retinopathy, hyperglycemia, pathogenesis, energy markers, experimental investigation

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## INTRODUCTION

Nowadays the problem of diabetes mellitus is one of the most pressing issues globally, given its prevalence, severity, and complications. The International Diabetes Federation reports that in 2023, diabetes was diagnosed in 463 million individuals, with 91% having type 2 diabetes. By 2045, the incidence of diabetes is projected to rise to 700 million people, accounting for over 10% of the entire population. The relentless increase in diabetes prevalence is linked to urbanization, an aging population, stress, a higher percentage of individuals with obesity, sedentary lifestyles, changes in food quality, and the rising availability of products containing hidden fats and carbohydrates, as well as genetically modified components [1, 2].

It is crucial to note that diabetes mellitus disrupts most metabolic processes, leading to an increased risk of tissue damage in the body, and potentially causing serious secondary complications. Due to the lack of timely patient consultations and late diagnosis of type 2 diabetes, by the time the diagnosis is established, 50% of patients already have complications associated with the development of micro- and macroangiopathies, among which diabetic retinopathy (DR) is quite common. This pathology is increasingly widespread globally, taking on the characteristics of a non-communicable epidemic [2, 3].

Vascular pathologies are characterized by tissue hypoxia, leading to a deficit in energy status. DR is marked by disruptions in metabolic processes with pathological implications. Therefore, energy supply processes in retinal cells play a crucial role. Mitochondria serve as the "powerhouse" of the cell, participating in metabolic processes and respiration. Mitochondrial dysfunction significantly impacts tissue homeostasis. One of the primary functions of mitochondria is the production of adenosine triphosphate (ATP) – a molecule that acts as an "energy reservoir" for cells and the entire organism. There are two main pathways through which cells produce ATP: glycolysis and oxidative phosphorylation [3, 4, 5].

The retina is one of the most metabolically active tissues in the body, consuming more oxygen than the brain. Significant changes in mitochondrial bioenergetics can disrupt the functioning of the electron transport chain in mitochondria [4].

## AIM

Study the features of the energetic functions of retinal mitochondria under conditions of chronic hyperglycemia to expand the understanding of the pathogenic mechanisms involved in the development of DR.

## MATERIALS AND METHODS

The experimental study was carried out on non-linear rats. Controls were intact rats that received distilled water intragastrically. A streptozotocin model was used to reproduce type 2 diabetes. This model makes it possible to reproduce the main pathogenetic links of type 2 diabetes in humans – impaired secretion and action of insulin and is characterized by the development of intolerance to carbohydrates, relative insufficiency of insulin secretion in response to elevated glucose levels, and preservation of the secretory response to non-glucose secretogens. For this purpose, streptozotocin (SigmaAldrich Chemie GmbH, Germany) was administered to rats once intravenously at a dose of 65 mg/kg. Streptozotocin solution was prepared in 0.1 M citrate buffer (pH 4.5). In order to reduce the diabetogenic effect of streptozotocin, 15 minutes before its administration, nicotinamide (Afton Pharma, India) was administered intraperitoneally at a dose of 230 mg/kg, which allows preserving up to 40% of pancreatic insulin reserves in experimental rats, due to which the animals develop a stable basal hyperglycemia [6].

Lactate activity was determined using a kinetic method based on the reduction of pyruvate in the presence of NADH. The lactate content was measured using a spectrophotometric method, which relies on the formation of a lactate-iron complex with a maximum absorption at 390 nm [7].

Pyruvate content was assessed using the Umbreit method, which is based on the formation of a hydrazone when pyruvic acid reacts with 2,4-dinitrophenylhydrazine, expressed in mmol/mg of protein [6, 7].

The levels of adenine nucleotides (ATP, ADP) were determined using high-voltage paper electrophoresis followed by spectrophotometry at wavelengths of 260 and 290 nm [7].

In working with animals we adhered to the International Code of Medical Ethics (Venice, 1983), the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), the "General Ethical Principles of Animal Experiments" adopted at the First National Congress on Bioethics (Kyiv, 2001), Directive 2010/63/EU of the European Parliament and Council on the protection of animals used for scientific purposes, and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" No. 440-IX dated January 14, 2020.

Statistical processing of the obtained results was carried out with the help of the "Statistica 10.0" program. The probability of differences between the indicators of the control and experimental groups was determined by Student's and Fisher's tests. The level of reliability was accepted at $p<0.05$.

## RESULTS AND DISCUSSION

A significant increase in lactate levels in serum was observed: on day 60 there was in 1.2 times ($p<0.05$), and on day 120 – in 1.8 times ($p<0.05$) compared to intact animals (Table 1). Additionally, significant differences were noted between the results obtained on days 60 and 120 of the experiment.

The level of pyruvate on day 60 also increased significantly in 1.2 times ($p<0.05$) compared to intact rats (0.245±0.016 mmol/L vs. 0.294±0.019 mmol/L); on day 120 this parameter rose in 1.4 times ($p<0.05$) respectively (0.352±0.026 mmol/L vs. 0.294±0.019 mmol/L).

We found that ATP concentration in serum on day 60 was 704.8±42.7 nmol/L, which was in 1.1 times lower than that of intact animals; on day 120 it was in 1.4 times lower ($p<0.05$). ATP levels also showed significant differences over the course of the study.

ADP levels on the day 60 decreased in 1.2 times ($p<0.05$) compared to the intact group (305.72±14.6 nmol/L vs. 361.06±17.4 nmol/L); on day 120 – in 1.3 times ($p<0.05$) respectively (270.8±15.2 nmol/L vs. 361.06±17.4 nmol/L).

When studying these parameters in the retina, similar changes were found as in serum (Table 2). The lactate level on day 60 increased in 1.4 times ($p<0.05$) compared to intact animals (7.98±0.73 mmol/g vs. 5.67±0.51 mmol/g); on day 120 – in 2.0 times ($p<0.05$) respectively (11.2±0.9 mmol/g vs. 5.67±0.51 mmol/g).

On day 60 the level of pyruvate was 0.241±0.018 mmol/g, which was in 1.2 times ($p<0.05$) higher than that of intact rats; on day 120 this parameter increased in 1.5 times ($p<0.05$) to 0.288±0.018 mmol/g compared to 0.198±0.016 mmol/g in the control group.

The concentration of ATP and ADP in the retina showed the following changes: a significant decrease in ATP and ADP was noted on day 60 with reductions in 1.3 times ($p<0.05$) and in 1.2 times ($p<0.05$), respectively, compared to the intact group (2.51±0.16 μmol/g vs. 3.33±0.21 μmol/g; 0.725±0.050 μmol/g vs. 0.847±0.067 μmol/g). On day 120 ATP and ADP levels were 1.84±0.14 μmol/g and 0.641±0.035 μmol/g, which were lower in 1.8 times ($p<0.05$) and in 1.3 times ($p<0.05$), respectively, compared to the intact group.

We experimentally established that under conditions of prolonged hyperglycemia, rats with diabetic retinopathy develop a hypoxic state in the retina, with

**Table 1.** Study of the mitochondria energy profile of in the serum of rats with experimental diabetic retinopathy (X±Sx)

| Indicator | Intact group (n=10) | Control group (n=20) | |
|---|---|---|---|
| | | On day 60 | On day 120 |
| Lactate, mmol/L | 2,61±0,19 | 3,25±0,21* | 4,57±0,30*/** |
| Pyruvate, mmol/L | 0,245±0,016 | 0,294±0,019* | 0,352±0,026* |
| ATP, nmol/L | 807,61±60,3 | 704,8±42,7 | 576,9±35,8*/** |
| ADP, nmol/L | 361,06±17,4 | 305,72±14,6* | 270,8±15,2*/** |

Notes:
1. * – p<0.05 compared to the intact group of animals;
2. ** – p<0.05 relative to the values obtained on day 60;
3. n – number of animals in the group.

**Table 2.** Study of the mitochondria energy profile of in the retina of rats with experimental diabetic retinopathy (X±Sx)

| Indicator | Intact group (n=10) | Control group (n=20) | |
|---|---|---|---|
| | | On day 60 | On day 120 |
| Lactate, mmol/g | 5,67±0,51 | 7,98±0,73* | 11,2±0,9*/** |
| Pyruvate, mmol/g | 0,198±0,016 | 0,241±0,018* | 0,288±0,018* |
| ATP, μmol/g | 3,33±0,21 | 2,51±0,16* | 1,84±0,14*/** |
| ADP, μmol/g | 0,847±0,067 | 0,725±0,050* | 0,641±0,035*/** |

Notes:
1. * – p<0.05 compared to the intact group of animals;
2. ** – p<0.05 relative to the values obtained on day 60;
3. n – number of animals in the group.

increased levels of biochemical markers such as lactate and pyruvate compared to intact animals [8]. At the same time, a significant decrease in ATP and ADP was observed in the animals with the modeled pathology.

Lactate is an indicator of bioenergetic hypoxia and one of the primary markers of mitochondrial dysfunction. It is considered an early prognostic marker, as its levels rise before other signs of oxygen deprivation. The accumulation of lactic acid occurs when glycolytic breakdown of glucose surpasses the oxidation of pyruvate in mitochondria, typically due to reduced oxygen availability and mitochondrial dysfunction. In this case, the organism "switches" to a less efficient (anaerobic) energy production pathway through glucose breakdown, generating ATP. Lactate is the main byproduct of this anaerobic process. A significant increase in lactate in the plasma indicates mitochondrial dysfunction [2, 4, 9].

Furthermore, the lactate/pyruvate ratio reflects the balance between glycolytic and oxidative metabolism of carbohydrates [10].

In our study, alongside identifying hypoxia in the retinas of rats with modeled DR, we observed a decrease in the levels of adenine nucleotide metabolites ATP and ADP in both the retina and serum of experimental animals. These nucleotides serve as markers of cellular energy potential. As known, ATP and ADP levels are important regulators of insulin secretion, which is stimulated by glucose levels. In aerobic cells, phosphorylation potential is regulated by mechanisms typical of mitochondrial metabolism, leading to compensatory changes in electron transport. Alterations in mitochondrial energy function can result in damage to the entire mitochondrial electron transport chain [4, 7, 10].

Thus, the results obtained from the DR model regarding the likely metabolic mechanisms underlying the protective action of the eye against the development of diabetic complications in the retina will be significant for further understanding the pathogenesis of diabetic retinopathy.

## CONCLUSIONS

1. The increase in lactate and pyruvate levels may indicate a reduction in the energy potential of the retina under conditions of experimental diabetic retinopathy, while the elevation of ATP and ADP activity suggests the activation of energy processes and the development of adaptation to hypoxia in retinal cells.
2. The analysis of lactate and pyruvate levels, as well as ATP and ADP, may serve as prognostic markers in predicting the severity of diabetic retinopathy in the context of prolonged hyperglycemia. These markers play an important role in understanding the pathogenic mechanisms underlying the development of retinopathy.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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