December 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.3 • 211-219

EFFECT OF MELATONIN ON THE CARBOHYDRATE METABOLISM IN THE HEART OF RATS WITH ALLOXAN DIABETES

Kushnir, O.Yu.*; Yaremii, I.M.

Higher State Educational Establishment of Ukraine «Bukovinian State Medical University»,
Teatralna sq. 2, Postal code 58002, Chernivtsi, Ukraine

*kushnir@bsmu.edu.ua

Abstract

The aim of this study was to determine the influence of melatonin on basal level of glucose in the blood (BG), glycogen content (GC), activities of glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase (PK) and lactate dehydrogenase (LDH) in the heart muscle tissues of alloxan diabetic rats under conditions of different photoperiod (artificial equinox, constant darkness and constant light). In the DM rats the LDH activity increased on average by 64%, whereas the GC and the activities of PK and G6PD decreased on average by 20%, 60%, and 47% respectively compared with control values. All of these changes were not dependent on the light conditions. The BG level of the IGT rats didn't reliably differ from the control, however, the LDH and the G6PD activities were respectively higher on average by 34% and 58%, except rats under the constant light conditions whose G6PD activity was lower by 48%. A melatonin administration (7 daily injections of 10 mg/kg) led to an improvement of the carbohydrate metabolism: the BG level, the GC, the activities of PK and LDH were normalized, while the G6PD activity was increased by an average of 30%. The influence of melatonin was more prominent in the IGT rats under the constant light conditions.

Keywords: melatonin; alloxan diabetes; carbohydrate metabolism; heart; rats.

Introduction

Hyperglycemia causes oxidative stress by free radical formation and lipid peroxidation in tissues and thus may cause damage in target organs. Melatonin produced by pineal gland was shown to be an effective antioxidant. Since heart is target organs for coronary atherosclerosis and ischemia, we have studied the role of melatonin against alloxan-induced heart toxicity in rats.

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine with potent multifunctional biological and pharmacological effects, both receptor dependent and receptor-independent, including antioxidant, anticancer, antitumor, anti-inflammatory, anti-aging, anti-diabetic, antiviral and neuroprotective activities. Melatonin mitigates tissue injury via modification of abnormalities in redox status and other biochemical markers. At the molecular level, a biological and pharmacological activity of melatonin is attributed to nuclear factor-κappa beta (NF-κB) inhibition, c-Fos overexpression and matrix metalloproteinases-3 (MMP-3) down-regulation, which are regulators of pro-inflammatory and pro-fibrotic cytokines. There are numerous scientific reports on melatonin therapeutic potential in treatment of asthma, infection respiratory diseases, chronic obstructive pulmonary disease, lung cancer, pleural cavity diseases and vascular pulmonary disease [1].

It was shown that 14-day introduction of melatonin to alloxan diabetic rats under conditions of constant darkness led to a decrease in the basal glycaemia level as well as a stabilization in the indices of the antioxidant defense disturbed by an absolute deficit of insulin [2].

Melatonin is a highly efficient scavenger of reactive oxygen species and it also exhibits beneficial anti-inflammatory and anti-ageing effects. Also, melatonin exhibits marked antioxidant and anti-ageing effects in skeletal muscles, similar to those of alpha-lipoic acid even if given in much lower doses [3]. But the relationship between the effects of melatonin on glycolysis and antioxidant protection in heart muscle of alloxan diabetic rats is poorly understood.

The aim of this study was to determine the influence of melatonin on basal glucose (BG) level in the blood, glycogen content (GC), glucose-6-phosphate dehydrogenase [EC 1.1.1.49] (G6PD), pyruvate kinase [EC 2.7.1.40] (PK) and lactate dehydrogenase [EC 1.1.1.27] (LDH) activities in the heart muscle of alloxan diabetic rats under conditions of different photoperiod.

Methods

The research was performed in compliance with the Rules of the work using experimental animals (1977) and the Council of Europe Convention on the Protection of Vertebrate Animals used in experiments and other scientific purposes (18 March 1986). The experiments were carried out on 158 sexually mature male albino rats with the body mass 0.18 – 0.20 kg. Alloxan diabetes was evoked via intraperitoneal injection with a 5% solution of alloxan monohydrate in a dose of 170 mg/kg of body weight. The animals were divided into 3 groups: 1) rats under artificial equinox (Light: Darkness = 12:12, LD); 2) rats under constant dark (L:D = 0:24, DD); 3) rats under constant light (L:D = 24:0, LL). In each group there were 5 subgroups: 1) control group; 2) group with DM (BG level ≥ 8.0 mmol/l); 3) alloxan diabetic animals with overt diabetes which were injected with melatonin; 4) alloxan diabetic rats with impaired glucose tolerance (IGT) (BG level ≤ 6.9 mmol/l); 5) alloxan diabetic animals with IGT which were injected with melatonin. Melatonin ("Sigma", USA) was injected intraperitoneally in a dose of 10 mg/kg of body weight at 8 a. m. daily over 7 days starting with a 5th 24-hour period after the alloxan injection. The blood was taken from the tail vein to evaluate the BG level using "OneTouchUltra" ("LifeScan", USA). The rats were sacrificed at the 12^{th} day of the experiments in accordance with the ethical treatment of animals. To precipitate glycogen heart tissue was split with 30% KOH solution followed by the addition of ethanol and cooling. Then, glycogen was hydrolyzed with sulfuric acid to glucose, the level of which was used as an indicator of the GC. To determine the enzymes activities by standart methods [4] heart tissue was quickly removed, rinsed in saline, blotted, weighed and homogenized. Then the homogenate (5% in ice-cold 0.25 mM tris-HCl-buffer) was ultracentrifugated (10 min at 1500r/min) and the supernatant was used for measurements.

Statistical analysis was performed using Statistica 10 (StatSoft Inc). Prior to analysis, Shapiro-Wilk test was used to assess the normality of the

group data. According to the criterion, the samples distributions differed from normal distribution. Given these, use of the Mann-Whitney test was considered sufficient for valid conclusions to be made. Differences were considered to be statistically significant if $P \leq 0.05$

Results

The BG level in the animals under LL conditions tended to increase by 12% from baseline over a 1-week period starting on the 4th day of the experiments. Melatonin insertion reduced (but not normalized as under DD and LD conditions) this level 1.6 times compared with DM animals, indicating its hypoglycemic action in LL conditions is less pronounced. The prevalence of diabetes has exponentially increased in recent decades due to such factors as nocturnal lifestyle and ageing, both of which influence the amount of melatonin produced in the pineal gland [5].

In the heart, the most abundant glucose transporters are GLUT1 and GLUT4. GLUT1 mainly localizes on plasma membrane, and is responsible for a significant component of basal cardiac glucose uptake. On the other hand, GLUT4 is mostly present in the intracellular vesicles at resting stages, and is translocated to the plasma membrane upon insulin stimulation. GLUT4-mediated glucose transport represents an important mechanism by which the net flux of glucose uptake by the cell can be tightly regulated by environmental changes. [6]. Insulin depletion induces the down-regulation of GLUT4 in the heart. In most rodent models of diabetes (type 1 or type 2), consistent observations have demonstrated that the expression of GLUT4 is decreased in the heart, associated with the decline in glucose utilization in the heart.

In adipocytes, GLUT4 can be phosphorylated at its C-terminal end in response to catecholamine stimulation. This phosphorylation does not affect GLUT4 translocation to plasma membrane, but is associated with decreases glucose uptake. Several stimuli including insulin, ischemia, exercise, and catecholamine have been well documented to mediate GLUTs translocation in the heart. Similar to adipose tissue and skeletal muscle, the translocation of GLUT4 transporters accounts for the majority of the transarcolemmal transport of glucose in cardiomyocytes.

Wortmannin, the inhibitor of phosphoinositide-3-kinase (PI3K) can block insulin stimulated glucose transport but failed to block the translocation of GLUTs in response to myocardial ischemia or skeletal muscle contraction [6].

A recent study, however demonstrates that in neonatal cardiomyocytes blocking calcium response in the presence of insulin is sufficient to reduce insulin-stimulated glucose uptake and to prevent GLUT4 translocation, suggesting that the effect of insulin on glucose transport can be partially mediated by calcium [7]. On the other hand, calcium is not required for 2,4-dinitrophenol mediated GLUT4 translocation [8], indicating that calcium is not required for metabolic stress mediated glucose transport.

Chronic increases in myocardial glucose uptake and utilization reduces the metabolic flexibility and renders the heart susceptible to contractile dysfunction in high fat diet induced obesity [9]. Another study using an inducible overexpression model shows that short-term cardiac specific induction of GLUT1 at the onset of pressure overload preserves mitochondrial function and attenuates pathological remodeling, but exacerbates the hypertrophic phenotype and is insufficient to prevent pressure overload-induced cardiac contractile dysfunction [10].

Breakdown of glycogen provides a critical source of fuel during acute increases of cardiac work, for example, during adrenergic stimulation and intensive exercise, or during ischemia [11].

Diabetes may cause myocardial cell damage and eventually lead to the development of diabetic cardiomyopathy (DCM). DCM is a disease caused by diabetes that is independent of coronary artery disease, hypertension and heart valve disease. The main characteristics of DCM include oxidative stress, cardiac hypertrophy, apoptosis, myocardial fibrosis and impaired cardiac function. Melatonin, a potent antioxidant agent, is essential for glucose homeostasis and regulation.

We have established reduction of glycogen (Fig. 1) in the heart of diabetic animals in average by 20% compared with the control. Such changes are likely occurred because of a decrease in revenues of glucose in heart muscle tissue and inhibition of its use. According to our research, week daily administration of melatonin to diabetic rats at 10

mg/kg of b.w. resulted in normalization of heart muscle glycogen content. The positive impact of melatonin probably mediated by improved of glucose utilization due to increased capture of tissues and activating major enzymes of glycogenesis.

The activity of PK and G6PD decreased on average by 60%, and 47% respectively (Fig. 2, 3). It is well known that heart muscle tissue is dependent on the presence of insulin. Pentose Phosphate Pathway (PPP) provides an alternative fate for glycolytic intermediates. PPP contains the oxidative phase and the nonoxidative phase characterized by a series of enzymatic reactions (Fig. 3). Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme that controls the entry of G-6-P into the PPP. In oxidative phase of PPP, G6PD oxidizes G-6-P generated from the initial reaction of glycolysis to produce 6-phosphogluconolactone and one molecules of NADPH. In the nonoxidative phase, ribulose-5-phosphate may be used for nucleotide synthesis, especially important for cell proliferation and growth. It also can be used for aromatic amino acid synthesis or converted to F-6-P and glyceraldehydes-3-phosphate through a series of aldolases and transketolases that reenter the pathway or are oxidized as fuel [12]. NADPH generated from the oxidative PPP is used for maintaining the reduced glutathione level, an important element for cellular antioxidative defense [13]. This reaction is generally considered beneficial, since deficiency of G6PD causes cells more susceptible to oxidative damage [14]. The capacity of the oxidative PPP in the heart is limited as the basal activity of G6PD is very low [15]. Collectively, these observations suggest that G6PD is a critical component of the cellular antioxidant system in adult cardiomyocytes, and furthermore, that oxidative PPP plays an essential role in regulating cardiac function through maintaining the capacity of the antioxidant glutathione system.

Pyruvate kinase catalyzes the conversion from phosphoenolpyruvate to pyruvate and generates one molecule of ATP, another irreversible step of glycolysis in the heart (Fig. 3). Oxidation of pyruvate in the mitochondria completes the process of glucose oxidation. A highly regulated step in pyruvate oxidation is the irreversible conversion of pyruvate to acetyl coenzyme A (acetyl-coA) by pyruvate dehydrogenase (PDH) complex. Phosphorylation of PDH by PDH kinase (PDK) inhibits its activity and it is reactivated by dephosphorylation. PDK is activated by elevated ratios of acetyl-CoA/free CoA or NADH/NAD+ and is inhibited by pyruvate [16]. If not oxidized via PDH, pyruvate can form lactate via lactate dehydrogenase (LDH) reaction coupled with consumption of an electron from the NADH. This reaction is important for cellular redox state, especially under conditions of reduced oxidative metabolism. Alternatively pyruvate can be converted to oxaloacetate or malate catalyzed by pyruvate carboxylase or malic enzyme (ME). The so-called anaplerotic reaction contributes to maintain the pool size of TCA intermediates and TCA function in the heart. Pyruvate can also contribute to anaplerosis by transamination with glutamate to form alanine and the TCA cycle intermediate alpha-ketoglutarate [17].

If pyruvate cannot be oxidized, it can be converted to lactate through LDH. Although the LDH reaction is considered to be near equilibrium, reduced availability of pyruvate for oxidation might lead to exaggerated conversion of cytoplasmic pyruvate into lactate in cardiac hypertrophy due to the elevated LDH activity. Consistently shown in experimental and human hypertrophied hearts, increased activity of LDH has been documented [18]. Several studies also observe that LDH isoform expression shifts toward the muscle-type subunit in hypertrophied hearts, with elevated lactate production. Although the production of lactate is increased in hypertrophied hearts, the overall rate of lactate oxidation is similar to nonhypertrophied hearts [19]. It is possible that there is reduced availability of pyruvate to oxidative metabolism in mitochondria due to the aberrant LDH activity in the hypertrophied heart. Under all types of illumination the activity of LDH (Fig. 4) increases on average by 64% in the heart tissues of alloxan diabetic rats with DM compared with the control value.

The level of BG in the IGT rats under all types of illumination didn't reliably differ from the indices of intact rats. However, the activities of G6PD (Fig. 2) and LDH (Fig. 4) were, on average, higher by 58% and 34% respectively than in the control groups. The GC (Fig. 1) and the activity of PK (Fig. 3) in heart of the IGT rats show tendency to decrease by an

average of 12% from control under different light conditions, except in rats with IGT under LL conditions whose indices of G6PD (Fig. 2) were lower by 48% than those in the control group. The injection of melatonin in a dose of 10 mg/kg was conducive to a normalization of the indices of carbohydrate metabolism in the group of animals with IGT in different light conditions. Additionally [20], exposure to light at night and ageing, both of which lower endogenous melatonin levels, may contribute to the incidence and/or development of diabetes.

A 7-day injection of the same dose of melatonin to the DM rats contributed to a normalization of the BG level, the GC (Fig. 2), the activities of PK (Fif. 3) and LDH (Fig. 4) as well as to a considerable increase of the G6PD activity (Fig. 1) whose level exceeded the control value by an average of 30%. Under the influence of melatonin, the increased G6PD activity may be due to an increased amount of substrate for G6PD (stimulating flow of glucose into cells and its phosphorylation) and direct action [21].

Discussion

Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular [22].

Melatonin, a potent antioxidant agent, is essential for glucose homeostasis and regulation [23]. It was determined [7] that melatonin supplementation influenses oxidative stress parameters in elderly NIDDM patients. Moreover, earlier [24, 25] we investigated Langergans islends in diabetic rats and recorded histomorphological alterations: their pancreatic share reliably decreased by 55%, number and percentage of beta-cells with necrosis decreased by 90% and 97% respectively compared with the control. Melatonin treatment caused a sharp decrease in the elevated serum glucose and partial regeneration/proliferation of beta-cells. It was concluded that the hypoglycemic action of melatonin could be partly due to amelioration in beta-cells of pancreatic islets.

Melatonin stimulates glucose transport to skeletal muscle cells via insulin receptor substrate-1/phosphoinositide-3-kinase pathway, which implies, at the molecular level, its role in glucose homeostasis and possibly in diabetes. Endogenous melatonin level may contribute to the incidence and/or development of diabetes. A strong phosphorylation of inositol-requiring enzyme 1 (IRE-1), c-JUN NH2-terminal kinase (JNK) and insulin receptor substrate 1 (IRS-1) serine as well as a dramatic decrease of IRS-1 tyrosine phosphorylation were observed in the presence of tunicamycin. All of these lead to a blockage of insulin signaling in skeletal muscle cells, which is reversed by a melatonin pretreatment. Also, melatonin may increase a plasma concentration of leptin in mice [26] that terminally ill insulin-deficient rodents with uncontrolled diabetes due to autoimmune or chemical destruction of beta-cells were made hyperleptinemic by an adenoviral transfer of the leptin gene. Within approximately 10 days their severe hyperglycemia and ketosis were corrected. Despite the lack of insulin, moribund animals resumed linear growth and appeared normal. Normoglycemia persisted 10-80 days without other treatment while normal physiological conditions lasted for approximately 175 days despite reappearance of moderate hyperglycemia. Inhibition of gluconeogenesis by suppression of hyperglucagonemia and reduction of hepatic cAMP response element-binding protein, phoshoenolpyruvate carboxykinase and peroxisome proliferator-activated receptor-gamma-coactivator-1alpha may explain the anticatabolic effect. Up-regulation of insulin-like growth factor 1 (IGF-1) expression, plasma levels and increasing IGF-1 receptor phosphorylation in muscles may explain the increased insulin receptor substrate 1, PI3K and ERK phosphorylation in skeletal muscles. These findings [26] suggest that leptin reverses the catabolic consequences of a total lack of insulin, potentially by suppressing glucagon action on the liver and enhancing the insulinomimetic actions of IGF-1 on skeletal muscle, and suggest strategies for making type 1 diabetes insulin-independent.

Here, it has been ascertained that alloxan monohydrate administration results in a significant elevation of the blood basal glycemia level and an increase of the activities of lactate dehydrogenase. However, a decrease of the GC and the G6PD activity in heart tissue were directly dependent on the presence of hyperglycemia. It can be concluded that the administration of melatonin notably recovered the heart from hyperglycemia induced antioxidant imbalance, inflammation and apoptosis

as well as rectified the imbalance in carbohydrate metabolism.

References

1. Nabavi SF, Habtemariam S, Daglia M, Sureda A, Sobarzo-Sánchez E, Selamoglu Z, Gulhan MF, Nabavi SM. Melatonin and respiratory diseases: a review. Curr Top Med Chem. 2017;17(4):467-488.
2. Krishna T, Yaremii I, Kushnir O. Protective effects of melatonin in alloxan diabetic rats under conditions of constant darkness. Book of abstracts of 24-th European Students' Conference ["Exploring the unknown"]. 2013; ESCID 396.
3. Favero G, Rodella LF, Nardo L, Giugno L, Cocchi MA, Borsani E, Reiter RJ, Rezzani R. A comparison of melatonin and α-lipoic acid in the induction of antioxidant defences in L6 rat skeletal muscle cells. Age (Dordr). 2015;37(4):9824.
4. Kushnir O.Yu., Yaremii I.M., Shvetsv V.I., Shvets N.V. Influence of melatonin on carbohydrate metabolism in the kidney of alloxan diabetic rats. Fiziol. journ., 2017;63(4):64 – 71.
5. Kushnir A., Yaremii I, Malinevskaya A. Impact of melatonin on the activity of pyruvate kinase in the liver of alloxan – diabetic and tetrachlormethane intoxicated rats exposed to light deprivation. European Applied Sciences. 2014;2:35 – 37.
6. Dan Shao, Rong Tian. Compr Physiol. Glucose Transporters in Cardiac Metabolism and Hypertrophy. Compr Physiol. 2015; 6(1): 331–351.doi: 10.1002/cphy.c150016
7. Contreras-Ferrat AE, Toro B, Bravo R, Parra V, Vasquez C, Ibarra C, Mears D, Chiong M, Jaimovich E, Klip A, Lavandero S. An inositol 1,4,5-triphosphate (IP3)-IP3 receptor pathway is required for insulin-stimulated glucose transporter 4 translocation and glucose uptake in cardiomyocytes. Endocrinology. 2010;151:4665–4677.
8. Pelletier A, Joly E, Prentki M, Coderre L. Adenosine 5′-monophosphate-activated protein kinase and p38 mitogen-activated protein kinase participate in the stimulation of glucose uptake by dinitrophenol in adult cardiomyocytes. Endocrinology. 2005;146:2285–2294.
9. Yan J, Young ME, Cui L, Lopaschuk GD, Liao R, Tian R. Increased glucose uptake and oxidation in mouse hearts prevent high fatty acid oxidation but cause cardiac dysfunction in diet-induced obesity. Circulation.2009;119:2818–2828.
10. Pereira RO, Wende AR, Olsen C, Soto J, Rawlings T, Zhu Y, Anderson SM, Abel ED. Inducible overexpression of GLUT1 prevents mitochondrial dysfunction and attenuates structural remodeling in pressure overload but does not prevent left ventricular dysfunction. J Am Heart Assoc. 2013;2:e000301.
11. Hecker PA, Leopold JA, Gupte SA, Recchia FA, Stanley WC. Impact of glucose-6-phosphate dehydrogenase deficiency on the pathophysiology of cardiovascular disease. Am J Physiol Heart Circ Physiol. 2013;304:H491–H500.
12. Efferth T, Schwarzl SM, Smith J, Osieka R. Role of glucose-6-phosphate dehydrogenase for oxidative stress and apoptosis. Cell Death Differ. 2006;13:527–528. author reply 529–530.
13. Fico A, Paglialunga F, Cigliano L, Abrescia P, Verde P, Martini G, Iaccarino I, Filosa S. Glucose-6-phosphate dehydrogenase plays a crucial role in protection from redox-stress-induced apoptosis. Cell Death Differ. 2004;11:823–831.
14. Jain M, Brenner DA, Cui L, Lim CC, Wang B, Pimentel DR, Koh S, Sawyer DB, Leopold JA, Handy DE, Apstein CS, Liao R. Glucose-6-phosphate dehydrogenase modulates cytosolic redox status and contractile phenotype in adult cardiomyocytes. Circulation. 2003;108:173–173.
15. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev. 2005;85:1093–1129.
16. Des Rosiers C, Labarthe F, Lloyd SG, Chatham JC. Cardiac anaplerosis in health and disease: food for thought. Cardiovasc Res. 2011;90:210–219.

17. Contreras-Ferrat AE, Toro B, Bravo R, Parra V, Vasquez C, Ibarra C, Mears D, Chiong M, Jaimovich E, Klip A, Lavandero S. An inositol 1,4,5-triphosphate (IP3)-IP3 receptor pathway is required for insulin-stimulated glucose transporter 4 translocation and glucose uptake in cardiomyocytes. Endocrinology. 2010;151:4665–4677.
18. Lin GJ, Huang SH, Chen YW, Hueng DY, Chien MW, Chia WT, Chang DM, Sytwu HK. Melatonin prolongs islet graft survival in diabetic NOD mice. J Pineal Res. 2009 Oct;47(3):284-92.
19. Aly HF, Mantawy MM. Comparative effects of zinc, selenium and vitamin E or their combination on carbohydrate metabolizing enzymes and oxidative stress in streptozotocin induced-diabetic rats. Eur Rev Med Pharmacol Sci. 2012;16(1):66-78.
20. Gerush I, Boichuk T, Yaremii I, Kushnir O, Gerush O. Effects of melatonin on the glutathione system in the blood of alloxan diabetic rats. The International Union of Biochemistry and Molecular Biology (IUBMB) and The Federation of European Biochemical Societies (FEBS). 2012;279(1):88.
21. Kushnir A., Meshchyshen I, Yaremii I. Hypoglycemic and antioxidant action of melatonin in alloxan diabetic rats. Annales Universitatis Marie Curie Skladovska. 2010;23(3,31): 227 – 230.
22. Czuczejko J, Sielski Ł, Woźniak B, Woźniak A, Szewczyk-Golec K. Melatonin supplementation improves oxidative and inflammatory state in the blood of professional athletes during the preparatory period for competitions. Free Radical Research. 2019;53(2):198-209.
23. Brar J, Chhokar A. Kushnir. O. Influence of melatonin long term introduction on condition of the Langergans islets of the pancreas i alloxan diabetic rats. 22-nd Inter. Stud. Congress of biomedical sciences. Book of abstract. 2015; 544.
24. Kushnir A, Davydenko I. Influence of melatonin on condition of the langergans isles of the pancreas in alloksan diabetic rats. World of Medicine and Biology. 2009;5(4):31-35.
25. Buonfiglio D, Parthimos R, Dantas R. Melatonin Absence Leads to Long-Term Leptin Resistance and Overweight in Rats Front Endocrinol (Lausanne). 2018; 9: 122.
26. Oberlin D and Buettner C. How does leptin restore euglycemia in insulin-deficient diabetes? J Clin Invest. 2017;127(2):450–453.

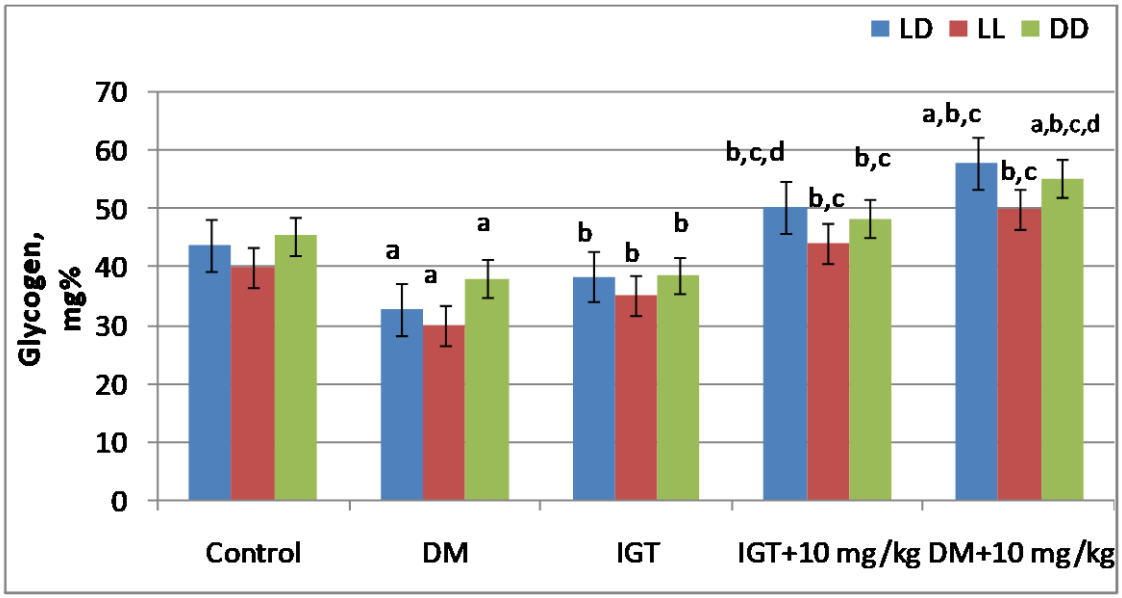

Figure 1. Changes in glycogen content in heart of rats, (n=6, x±Sx) : 1. a, b, c, d - changes are reliable (p≤0.05). 2. a - concerning control; b - concerning rats with DM; c – concerning rats with IGT; d – concerning the values of control animals in the conditions of natural (artificial) equinox - LD.

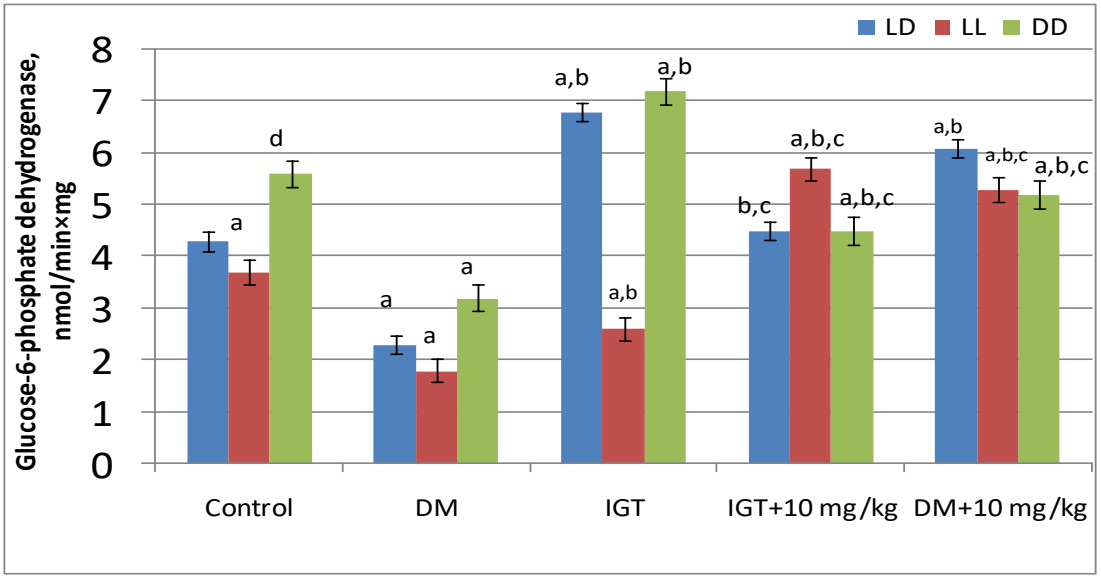

Figure 2. Changes of glucose-6-phosphate dehydrogenase activity in heart of rats, (n=6, x±Sx) : 1. a, b, c, d - changes are reliable (p≤0.05). 2. a - concerning control; b - concerning rats with DM; c – concerning rats with IGT; d – concerning the values of control animals in the conditions of natural (artificial) equinox - LD.

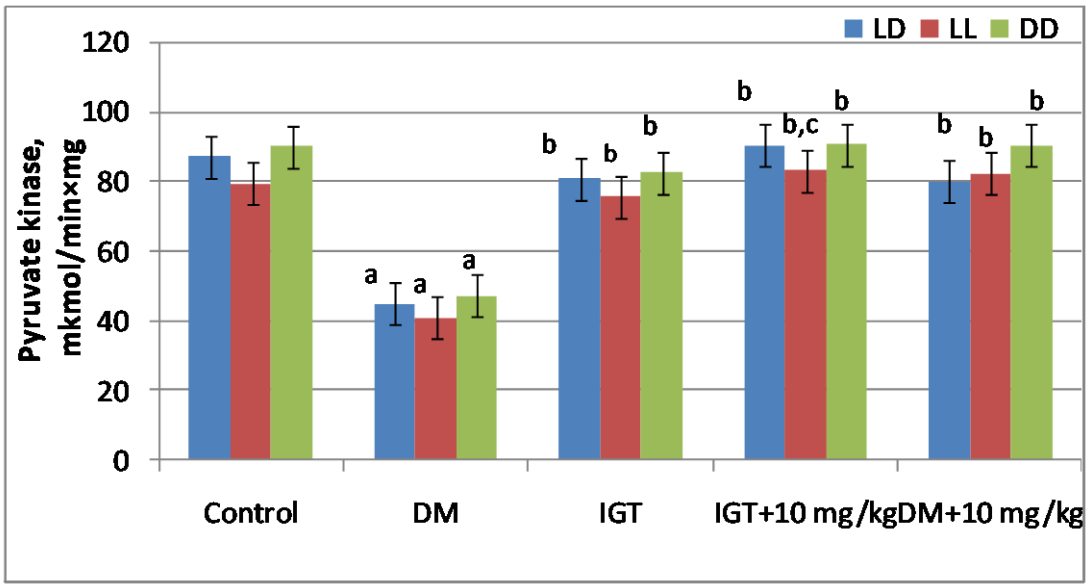

Figure 3. Changes of pyruvate kinase activity in heart of rats, (n=6, x±Sx) : 1. a, b, c, d - changes are reliable (p≤0.05). 2. a - concerning control; b - concerning rats with DM; c – concerning rats with IGT; d – concerning the values of control animals in the conditions of natural (artificial) equinox - LD.

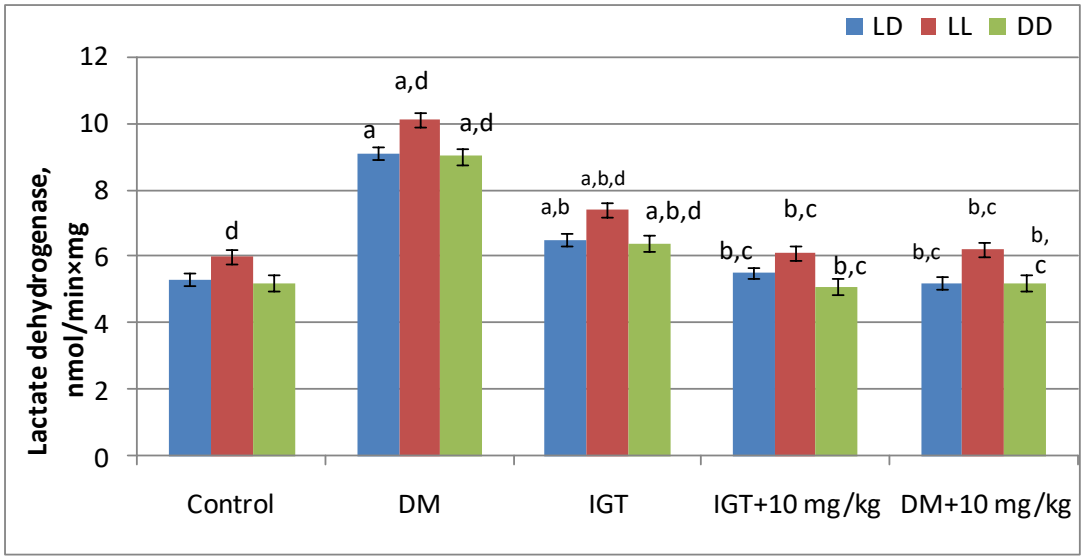

Figure 4. Changes of lactate dehydrogenase activity in heart of rats, (n=6, x±Sx) : 1. a, b, c, d - changes are reliable (p≤0.05). 2. a - concerning control; b - concerning rats with DM; c – concerning rats with IGT; d – concerning the values of control animals in the conditions of natural (artificial) equinox - LD.