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Transcriptional regulation of lactate dehydrogenase activity in rat kidney cells in diabetic nephropathy

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ABSTRACT

Aim. To study the features of transcriptional regulation of the activity and isoenzyme composition of lactate dehydrogenase in the kidneys of *Rattus norvegicus* L. in diabetic nephropathy.

Materials and methods. The study included 20 male laboratory rats (*Rattus norvegicus* L.) divided into two equal groups: "Norm" – intact rats injected with 0.9% NaCl intraperitoneally and "Diabetes" – animals with alloxan-induced diabetes (DM1 model). The activity, subcellular localization, and mobility of lactate dehydrogenase (LDH, EC 1.1.1.27) isoenzymes were studied using spectrophotometry and electrophoresis. *LDHA* and *LDHB* gene transcripts were analyzed by the polymerase chain reaction.

Results. Analysis of the LDH activity showed that this parameter increased by more than 6 times in the animals with diabetic nephropathy compared to the control group. Moreover, the increase in the rate of the LDH activity was a consequence of the enzyme activation in all the studied compartments of the cell and is consistent with the parameter in the homogenate. The increase in the LDH activity in diabetic nephropathy may result from redistribution of the activity rate between the available isoforms and may be associated with an increase in the transcription rate of genes encoding subunits A and B of this enzyme.

Conclusion. The increase in the LDH activity is likely associated with the activation of renal gluconeogenesis, the main substrate for which is lactic acid reabsorbed in the renal glomeruli. The revealed increase in the LDH activity in the kidneys of rats with diabetic nephropathy may be associated with adaptation of their metabolism to the pathological state.

Keywords: lactate dehydrogenase, diabetic nephropathy, isoenzyme, regulation, transcription

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. The study was approved by the Ethics Committee for Biomedical Research at Voronezh State University (Protocol No. 42-04 of 05.09.2022).

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Транскрипционная регуляция функционирования лактатдегидрогеназы в клетках почек крыс при диабетической нефропатии

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РЕЗЮМЕ

Целью данной работы являлось изучение особенностей транскрипционной регуляции активности и изоферментного состава лактатдегидрогеназы в почках *Rattus norvegicus* L. при диабетической нефропатии.

Материалы и методы. Проведено исследование 20 самцов лабораторных крыс *Rattus norvegicus* L., разделенных на две равные группы: «Норма» – интактные крысы, которым внутрибрюшинно вводили 0,9%-й NaCl, и «Диабет» – животные с аллоксановым диабетом. Исследовалась активность, субклеточная локализация и подвижность изоферментов лактатдегидрогеназы (ЛДГ, КФ 1.1.1.27) с использованием спектрофотометрических, электрофоретических методов, а также использовалась полимеразная цепная реакция в реальном времени для анализа транскриптов генов *LDHA* и *LDHB*.

Результаты. Анализ активности ЛДГ показал, что данный параметр вырос более чем в 6 раз у животных с диабетической нефропатией по сравнению с контрольной группой. Возрастание скорости функционирования ЛДГ является следствием активизации фермента во всех исследуемых компартментах клетки и согласуется с показателем в гомогенате. Вероятно, увеличение активности ЛДГ при диабетической нефропатии является следствием перераспределения скорости функционирования между имеющимися изоформами и связано с усилением скорости транскрипции генов, кодирующих субъединицы А и В данного фермента.

Заключение. Усиление работы ЛДГ, вероятно, является следствием активизации почечного глюконеогенеза, основным субстратом для которого является именно молочная кислота, реадсорбируемая в почечных клубочках. Выявленное увеличение активности ЛДГ в почках крыс при диабетической нефропатии может быть связано с адаптацией их биохимического метаболизма к патологическому состоянию.

Ключевые слова: лактатдегидрогеназа, диабетическая нефропатия, изофермент, регуляция, транскрипция

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Diabetes mellitus is one of the most important medical, social, and economic problems worldwide [1]. The main danger of this pathology is associated with the development of various complications, which include diabetic nephropathy (DN). Every year the number of people with diabetes mellitus grows, therefore, the risk of developing this disease increases for each person [2]. The mechanism of DN is based on the development of sclerotic changes in the renal glomeruli, which lead to impaired kidney function and chronic renal failure. The main problem of this pathology is that there are no pronounced symptoms at initial stages of diabetes mellitus. By the time the symptoms manifest and a diagnosis of DN is made, the disease is already progressing very actively and becomes practically incurable [3].

The kidneys maintain high resting metabolic rate in the human body [4] and occupy the second place in terms of oxygen consumption and mitochondrial content, after

the heart [5]. Such an active mechanism of energy production is crucial for maintaining normal kidney function, which requires active transport and reabsorption of dissolved substances, including amino acids, sugars and other essential elements, back into the blood. However, in DN caused by diabetes mellitus, damage to the kidney tissues is observed, which can cause metabolic changes in them, including activation of glycolysis and fatty acid metabolism, as well as mitochondrial dysfunction and impaired ATP production [6].

The kidneys contain lactate dehydrogenase (LDH, EC 1.1.1.27), which is involved in the final stage of anaerobic glycolysis and performs reversible conversion of pyruvate into lactate [7]. But there is very little information about the activity of this enzyme in DN, which is a relevant and interesting topic to study.

MATERIALS AND METHODS

The study included male laboratory rats (*Rattus norvegicus L)* weighing about 150–200 g (Stezar breeding station, Russia). The study was approved by the Ethics Committee for Biomedical Research at Voronezh State University (Protocol No. 42-04 of 05.09.2022). The study was performed in accordance with the principles of humanity set out in the European Council directives (86 / 609 / EEC) and the Declaration of Helsinki.

The DN model in type 1 diabetes mellitus was created by a single intraperitoneal injection of a 5% solution of alloxan diluted in 0.9% sodium citrate at a dose of 150 mg / kg of live weight [8]. The animals (n = 20) were randomly divided into 2 equal experimental groups: “Norm” – intact rats injected with 0.9% NaCl intraperitoneally, and “Diabetes” – animals with alloxan-induced diabetes. To control the incidence of diabetes mellitus in the experimental group of rats, the blood glucose level was determined using the glucose meter Sattelit Plus PKG-02.4. Blood sampling was performed from the caudal vein in the morning on an empty stomach. Creatinine clearance was assessed by the concentration of creatinine in the blood serum and urine using the Jaffe’s method using the Creatinine Vital kit (Vital Development Corporation LLC, Russia). The calculation was carried out according to the formula:

$$\text{Creatinine clearance}((\text{ml/min})/\text{kg}) = \frac{\text{urine volume in 24 h (ml)} \times \text{urine creatinine (mmol/l)}}{\frac{\text{serum creatinine (mmol/l)} \times 0.001}{\text{rat weight (kg)}}}$$

The anesthetized animals were decapitated 3 months after the administration of alloxan, and the kidneys were extracted. Kidney tissue was homogenized in a 10-fold volume of the isolation medium containing 1 mM EDTA; 2 mM KCl; 3 mM DTT; 0.35 M sucrose; and 50 mM Tris-HCl buffer (pH = 7.8). Centrifugation was carried out for 5 minutes at 3,000 g and a temperature of 4 °C. A supernatant (homogenate) was selected, which was later used to measure the activity of the enzyme. Cytoplasm and mitochondria were separated by differential centrifugation [9].

The cross-contamination assay was used to assess the purity of the studied fractions, measuring the activity of succinate dehydrogenase [10] and alcohol dehydrogenase [11].

LDH activity was measured by spectrophotometry at a wavelength of 340 nm. A decrease in the optical density of the solution associated with the utilization of NADH during the conversion of pyruvate to lactate in the spectrophotometry medium of the following composition was evaluated: 5 mM MgCl; 10 mM KCl; 1.25 mM NADH; 3 mM pyruvate; 10 mM potassium phosphate buffer, pH = 7.8.

Separation of LDH isoenzymes was performed by polyacrylamide gel electrophoresis at a temperature of 4 °C. For the gel to be seen, a tetrazolium salt-based method was used, which is based on the emergence of a blue-colored compound formazan, that is a product of HCT reduction [12].

The nucleotide sequences of the rat *LDHA* and *LDHB* mRNA genes were obtained from the international GenBank sequence database (https://www.ncbi.nlm.nih.gov/gene /?term=lactate+dehdrogenase+rat). Identification of gene homology and comparative analysis of their composition were carried out using the BLAST software (https://blast.ncbi.nlm.nih.gov /). The Primer- BLAST program located on the NCBI website was used for the selection of primers. The obtained sequences were tested for specificity to the desired genes in the Primer- BLAST and for the formation of crosslinking and other secondary structures in the ClustalOmega program. Specific primers for *LDHA* and *LDHB* rat LDH genes (*LDHA*: forward 5’-ctcagcgtcccatgtatcct-3’; reverse 5’-tgagatttcccccagaccac-3’; *LDHB*: forward 5’- ctggattctgctcggtttcg-3’; reverse 5’- tgaggtcagccacacttagg-3’) were selected based on the analyzed sequences.

RNA was isolated by phenol chloroform extraction [13], followed by visualization by agarose gel electrophoresis (1%) [14]. Reverse transcription was carried out in order to obtain cDNA using the M-MuLV reverse transcriptase enzyme and oligo(dT) primers (SibFerment, Russia) for the synthesis of the first cDNA chain according

to the manufacturer's instructions. The real-time polymerase chain reaction was carried out on the LightCycler 96 PCR analyzer (Roche, Switzerland). The Extra-mix for PCR HS-Taq PCR kits (DiaM, Russia) were used as reagents. SYBR Green I was taken as a dye. Amplification parameters: preliminary denaturation at 95 °C for 5 min, then a cycle: 95 °C – 20 s, 59 °C – 30 s, 72 °C – 40 s (detection), final elongation – 72 °C – 10 min.

To verify the reliability of the data obtained, all experiments and measurements were carried out using 8x biological and 5x analytical replicates. Calculations were carried out in the Microsoft Office Excel 2007 program, and their further analysis was performed in the Stattech program (StatTech v. 1.2.0; Stattech LLC, Russia). Quantitative variables were checked for normality of distribution using the Shapiro – Wilk test. The figures present the data as the mean and the standard error of the mean ($M \pm SE$). The results of the experiment were analyzed using the Student's t-test with the calculation of the mean and the standard deviation. The correlation analysis using the Pearson's correlation coefficient was performed to identify the relationships between the parameters. All the data presented in this paper are statistically significant, $p < 0.05$.

RESULTS

The data obtained when measuring the concentration of glucose in the blood of the rats are shown in Fig. 1. It demonstrates that in the rats included in the "Norm" group, the concentration of glucose in the blood fluctuated within 4.1 ± 1.3 mmol / l throughout the entire experiment, whereas in the animals exposed to prolonged alloxan intoxication, this parameter significantly exceeded normal values and was approximately 15.5 ± 2.7 mmol / l.

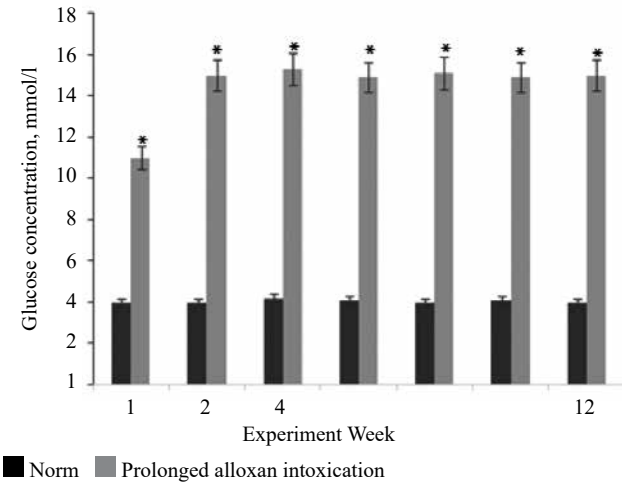

Fig. 1. Glucose concentration in the blood of experimental animals. Norm – healthy rats; Prolonged alloxan intoxication – animals with alloxan-induced diabetes. * $p < 0.01$. Here and in Fig. 2–4, 6, the method used is Student's *t*-test

A significant increase in blood glucose levels is the evidence of diabetes mellitus initiation.

We determined creatinine clearance in the blood serum of the experimental animals to assess glomerular filtration rate. The results presented in Fig.2 show that creatinine clearance increased by 1.64 times (3.05 and 1.83 ml / min / kg; $p < 0.05$) compared with the control group, which may indicate the development of an early stage of DN.

It was revealed that the level of protein excretion in the urine increased by 3.6 times by the second month of the experiment (from 8.1 to 28.9 mg / day; $p < 0.03$) (Fig. 3). At week 12 of the experiment, the value of the studied parameter decreased slightly and was 25.9 ± 0.1 mg / day. In the control group (rats injected with saline), the protein concentration in the urine fluctuated at the level of 7.89–8.01 mg / day, which corresponds to physiological values.

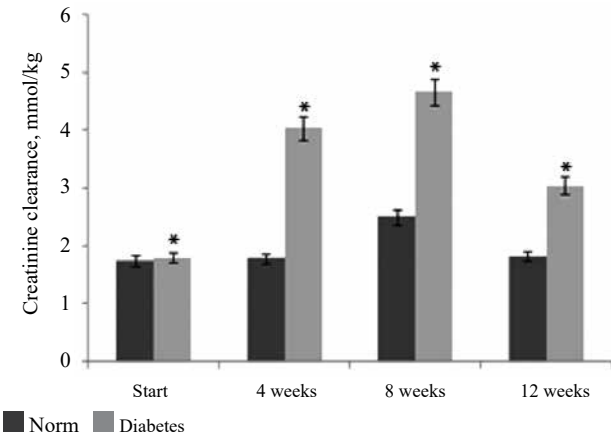

Fig.2. Determination of creatinine clearance in the blood serum of the animals with prolonged alloxan intoxication. Start – the beginning of the experiment; Norm – healthy rats; Diabetes – animals with alloxan-induced diabetes; * $p < 0.05$

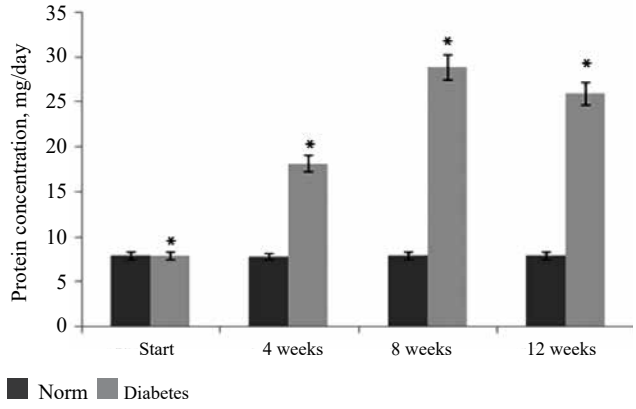

Fig. 3. Determination of protein concentration in the urine in the animals. Norm – healthy rats; Diabetes – animals with prolonged alloxan intoxication; * $p < 0.05$.

The analysis of the rate of LDH activity showed that this parameter increased by more than 6 times in the kidneys of the animals with DN compared with the control group (2.3 and 14.5 U / gram of wet mass; $p < 0.01$) (Fig. 4, *a*).

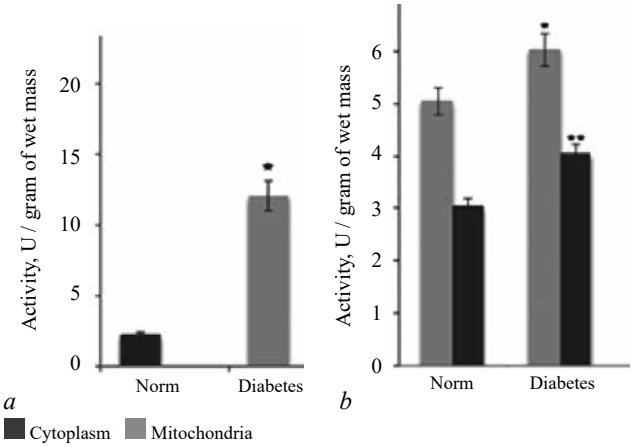

Fig. 4. Lactate dehydrogenase activity in the homogenate (*a*), cytoplasm and mitochondria (*b*) of rat kidneys. * $p < 0.01$; ** $p \leq 0.03$

LDH activity is observed both in the cytoplasm and mitochondria (Fig. 4, *b*), which is confirmed by the literature data on the subcellular localization of the enzyme under study.

Polyacrylamide gel electrophoresis conducted with subsequent manifestation of LDH activity showed the presence of 4 forms of the enzyme in the kidney cells of both groups of animals with R_f 0.04; 0.18; 0.26, and 0.32 (Fig. 5).

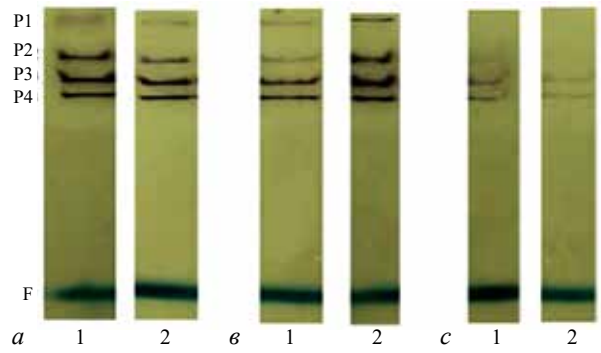

Fig. 5. Isoenzyme composition of lactate dehydrogenase in kidney cells of intact rats (1) and animals exposed to prolonged alloxan intoxication (2): *a* – homogenate; *b* – cytoplasmic fraction; *c* – mitochondria; P1–4 – protein bands; F – front line

A real-time polymerase chain reaction was performed to assess the expression level of genes encoding lactate dehydrogenase A and B subunits. As the analysis of the data obtained shows (Fig. 6), both genes are actively transcribed in the kidneys of rats with DN, but are less active in the kidneys of healthy animals. Moreover, with the development of this pathology, the expression of the *LDHA* gene increased by almost 3 times, and the expression of the *LDHB* gene – by more than 4 times.

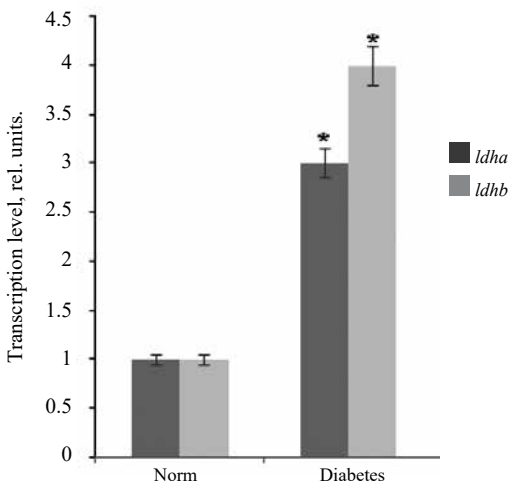

Fig. 6. Relative transcription level of *LDHA* and *LDHB* genes in the kidneys of healthy rats (Norm) and animals with diabetic nephropathy (Diabetes). * $p < 0.01$

DISCUSSION

The analysis of the results of blood and urine biochemistry in healthy rats and animals with prolonged alloxan intoxication showed that the latter have impaired kidney function against the background of diabetes mellitus. Thus, the data obtained confirm DN development in the experimental group of rats.

An increase in the LDH activity in the kidneys of the rats with DN may be associated with a need to utilize lactate entering the kidneys from the blood. It is known that in type 1 diabetes mellitus, there is a significant increase in renal gluconeogenesis, the main substrate for which is lactic acid reabsorbed in the renal glomeruli. This fact also explains the reason for the accumulation of glycogen in diabetic kidneys [15]. The LDH activity is observed both in the cytoplasm and in mitochondria, which is in line with the literature data on the subcellular localization of the enzyme under study [16].

A slight increase in the LDH activity in the mitochondria of the rats with alloxan-induced diabetes may be associated with the cell's need for additional energy to adapt the body to oxidative stress caused by alloxan administration. In addition, it is known that acceleration of mitochondrial oxidation of lactic acid is observed with intensive development of the nervous system, food deprivation, and physical overstrain [17]. The reliability of the data obtained was confirmed by the determination of cross-contamination with succinate dehydroge-

nase (mitochondrial enzyme) and alcohol dehydrogenase (cytoplasmic marker), which was approximately 11–14%. This fact testifies to the successful separation of factions.

Four isoforms of the enzyme were found in the kidney cells of both groups of animals when studying the isoenzyme composition of LDH. The increase in the LDH activity in DN may not be associated with the synthesis of additional forms of the enzyme, but is a consequence of the redistribution of the activity rate between the existing isoforms. Interestingly, in the cytoplasmic fraction of both intact (healthy) rats and animals with prolonged alloxan intoxication, the presence of four forms of the enzyme is also observed. At the same time, only two isoforms were found in the mitochondria.

The analysis of the level of gene expression revealed the relationship of this parameter ($r_s = 1$) with the LDH activity in rat kidney cells under normal and pathological conditions. The increase in the rate of LDH activity in the kidneys of the rats with prolonged alloxan intoxication is likely associated with the increase in the transcription rate of genes encoding subunits A and B of this enzyme.

CONCLUSION

Induction of prolonged alloxan intoxication allowed to determine a number of biochemical disorders (the level of protein excretion in the urine increased by 3.6 times (from 8.01 to 28.94 mg / day; $p < 0.03$), creatinine clearance – by 1.64 times (3.05 and 1.83 ml / min / kg; $p < 0.05$) compared with the control group of animals) associated with the development of DN against the background of high blood glucose. The analysis of the LDH activity showed that this parameter increased by more than 6 times in the kidneys of the animals with DN compared with the healthy animals.

It may be due to the increase in the concentration of transcripts of genes encoding subunits A and B of this enzyme. At the same time, no changes in the isoenzyme composition of LDH were detected. Increasing LDH activity may be necessary to activate renal gluconeogenesis, the main substrate for which is lactic acid reabsorbed in the renal glomeruli [17]. Thus, during the study, we revealed the increase in the rate of LDH activity in the kidneys of the rats with DN, which may be associated with the adaptation of their metabolism to the pathological condition.

REFERENCES

1. Schmidt A.M. Highlighting diabetes mellitus: the epidemic continues. *Arterioscler. Thromb. Vasc. Biol*. 2018;38(1):e1–e8 DOI: 10.1161/ATVBAHA.117.310221.
2. Shen Z., Fang Y., Xing T., Wang F. Diabetic nephropathy: from pathophysiology to treatment. *J. Diabetes Res*. 2017:2379432. DOI: 10.1155/2017/2379432.
3. Nagib A.M., Matter Y.E., Gheith O.A., Refaie A.F., Othman N.F., Al-Otaibi T. Diabetic nephropathy following posttransplant diabetes mellitus. *Exp. Clin. Transplant*. 2019;17(2):138–146. DOI: 10.6002/ect.2018.0157.
4. Silva P.H.I., Mohebbi N. Kidney metabolism and acid-base control: back to the basics. *Pflugers Arch*. 2022;474(8):919–934. DOI: 10.1007/s00424-022-02696-6.
5. Clark A.J., Parikh S.M. Mitochondrial metabolism in acute kidney injury. *Semin. Nephrol.* 2020;40(2):101–113. DOI: 10.1016/j.semnephrol.2020.01.002.
6. Samsu N. Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. *Biomed. Res. Int*. 2021;1497449. DOI: 10.1155/2021/1497449.
7. Osis G., Traylor A.M., Black L.M., Spangler D., George J.F., Zarjou A. et al. Agarwal expression of lactate dehydrogenase A and B isoforms in the mouse kidney. *Am. J. Physiol. Renal. Physiol.* 2021;320(5):F706–F718. DOI: 10.1152/ajprenal.00628.2020.
8. Ighodaro O.M., Adeosun A.M., Akinloye O.A. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina (Kaunas)*. 2017;53(6):365–374. DOI: 10.1016/j.medici.2018.02.001.
9. Djafarzadeh S., Jakob S.M. Isolation of intact mitochondria from skeletal muscle by differential centrifugation for high-resolution respirometry measurements. *J. Vis. Exp*. 2017;121:55251. DOI: 10.3791/55251.
10. Eprintsev A.T., Fedorin D.N., Selivanova N.V. Molecular aspects of the formation of the oligomeric structure in succinate dehydrogenase. Voronezh: Publishing House of the Central – Chernozem Region, 2016:263 (in Russ.).
11. Piechota J., Jelski W., Orywal K., Mroczko B. The comparison of total bile acid concentration and alcohol dehydrogenase activity as markers of intrahepatic cholestasis of pregnancy. *Acta Biochim. Pol.* 2021;69(1):173–176. DOI: 10.18388/abp.2020_5841.
12. Eprintsev A. T., Bondareva I. R., Selivanova N.V. Expression levels and activity of rat liver lactate dehydrogenase isoenzymes in alloxan-induced diabetes. *Biomedical Chemistry*. 2022;68(1):33–38 (in Russ.). DOI: 10.18097/PBMC20226801032.
13. Toni L.S., Garcia A.M., Jeffrey D.A., Jiang X., Stauffer B.L., Miyamoto Sh.D. et al. Optimization of phenol-chloroform RNA extraction. *MethodsX*. 2018;5:599–608. DOI: 10.1016/j.mex.2018.05.011.
14. Wittmeier P., Hummel S. Agarose gel electrophoresis to assess PCR product yield: comparison with spectrophotometry, fluorometry and qPCR. *Biotechniques*. 2022;72(4):155–158. DOI: 10.2144/btn-2021-0094.
15. Kume S., Araki S.I., Ugi S., Morino K., Koya D., Nishio Y. et al. Secular changes in clinical manifestations of kidney disease among Japanese adults with type 2 diabetes from 1996 to 2014. *Journal of Diabetes Investigation*. 2019;10(4):1032–1040. DOI: 10.1111/jdi.12977
16. Young A., Oldford C., Mailloux R. J. Lactate dehydrogenase supports lactate oxidation in mitochondria isolated from different mouse tissues. *Redox Biol*. 2020;28:101339. DOI: 10.1016/j.redox.2019.101339.

17. Glancy B., Kane D.A., Kavazis A.N., Goodwin M.L., Willis W.T., Gladden L.B. Mitochondrial lactate metabolism: history and implications for exercise and disease. *J. Physiol.* 202;599(3):863–888. DOI: 10.1113/JP278930.

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