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FEATURES OF IMMUNE METABOLISM OF LYMPHOCYTES IN PANCREATIC LYMPH NODES DURING EXPERIMENTAL STEPTOZOTOCIN-INDUCED DIABETES MELLITUS AND AFTER INTRODUCTION OF METFORMIN

ABSTRACT. **Background.** Metabolic changes in terms of developing diabetes, especially hyperglycemia, can directly affect immune metabolism of lymphocytes. **Objective:** The aim was to find out the level of mRNA gene expression of *Glut1*, *mTOR* and *AMPK1α* in pancreatic lymphatic nodes (PLN) in rats with experimental streptozotocin-induced diabetes mellitus (ESIDM) and after introduction of metformin. **Methods.** Glut1, mTOR and AMPK1α mRNA expression were analyzed by real-time reverse transcriptase-polymerase chain reaction. Total RNA was extracted from PLN tissue by Trizol RNA Prep 100 (Isogen, Russia), according to the manufacturer's instructions. RNA was re-suspended in RNase free water, quantified and subjected to RT-PCR reaction using RT-PCR kit; RT (Syntol, Russia). To determine the level of Glut1, mTOR and AMPK1α mRNA, RT-PCR was performed in real-time by thermal cycler CFX96 ™ Real-Time PCR Detection Systems (Bio-Rad Laboratories, Inc., USA). The relative level of gene expression were studied with rat reference genes GAPDH by the method ΔΔCt. Statistical analysis were conducted using available software «Bio-Rad SFX Manager 3.1» (Bio-Rad, USA). **Results.** It was established that hyperglycemia caused the transcript induction of genes of glucose transporter *Glut1* (in 9,9-28,9 times, $p<0,05$) and protein kinase *mTOR* (in 5.3-3.3 times, $p<0.05$) in cells of PLN. Introduction of metformin to the diabetic rats resulted in an increased level of mRNA gene of *AMPK1α*by on 87% ($p<0.05$) on the 3rd week and 38 fold ($p<0.05$) on the 5th week of ESIDM development and inhibition of *mTOR* expression in PLN (in 3-14.7 times, $p<0.05$). Revealed increase of *Glut1* and *mTOR* mRNA genes level in the PLN cells during diabetes can trigger their differentiation in effective pro-inflammatory subpopulation of Th1- and Th17-lymphocytes. **Conclusion.** Increased level of AMPK1α mRNA and inhibition of mTOR expression in PLN after *metformin* introduction to diabetic rats gives evidence about the possibility for correction of immune violations that develop during diabetes.

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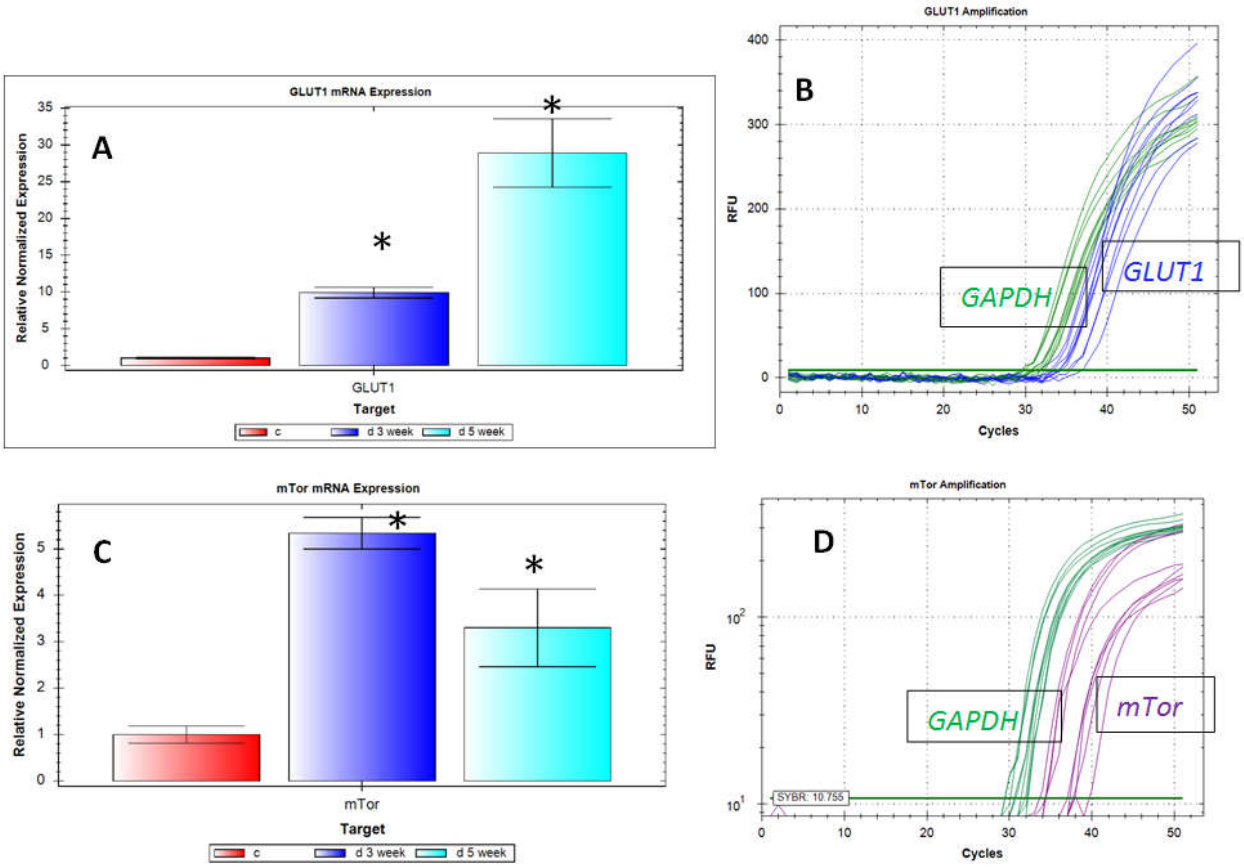

Fig. 1. Relative normalized number of genes *GLUT1* (A) and *mTOR* (C) mRNA with their amplification graphs (B, D) in the pancreatic lymph node cells. Normalization according to the method of ΔΔCt with reference gene *GAPDH*. c-control; d3, d5 – 3 weeks and 5 weeks of experimental steptozotocin-induced diabetes mellitus respectively.

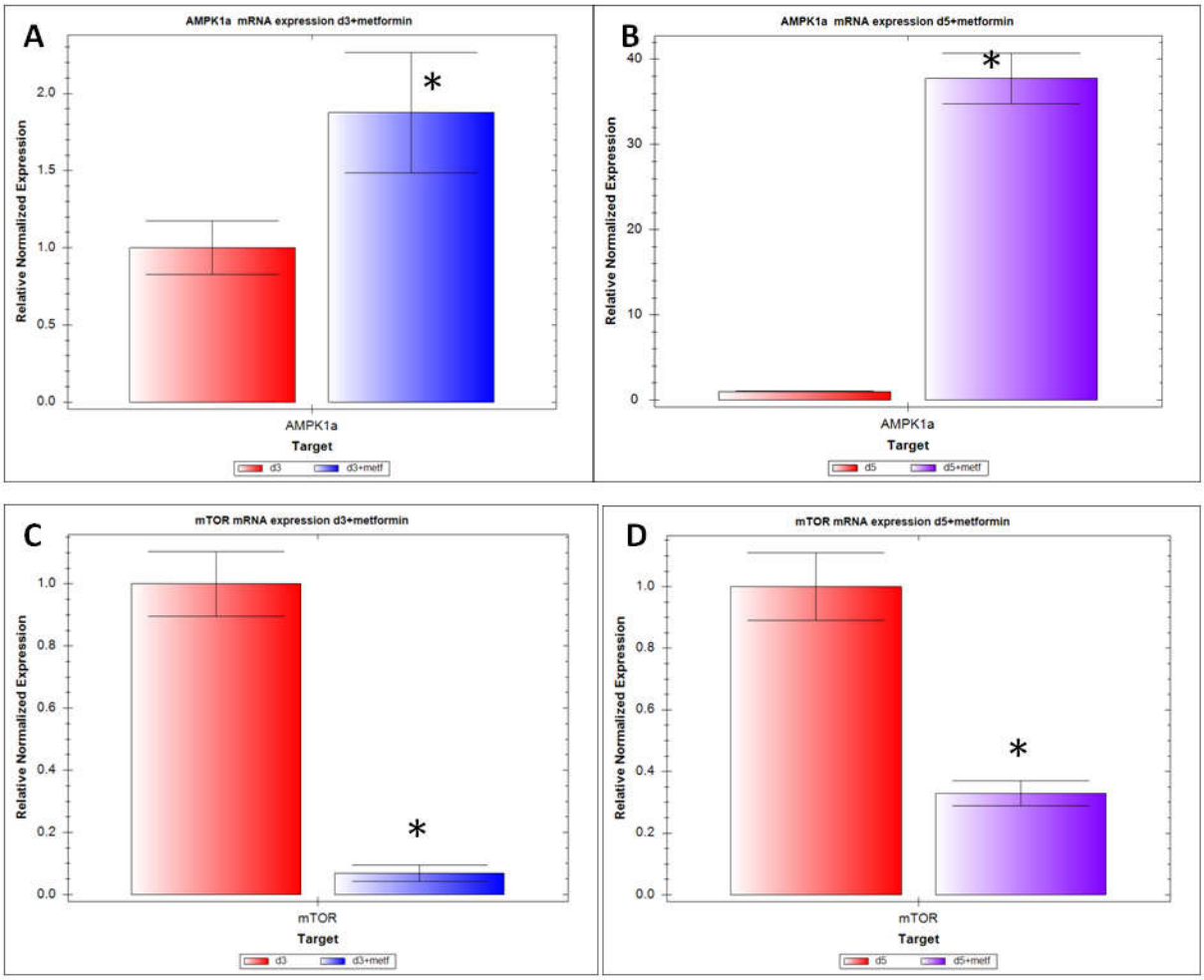

Fig. 2. Relative normalized number of genes *AMPK1α* (A,B) and *mTOR* (C, D) mRNA in the pancreatic lymph node cells after metformin introduction to diabetic rats. Normalization according to the method of ΔΔCt with reference gene *GAPDH*. c-control; d3, d5 – 3 weeks and 5 weeks of experimental steptozotocin-induced diabetes mellitus respectively.

Fig. 3. Immune metabolism of lymphocytes in diabetes mellitus.

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