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Effect of Exercise on GLUT4 Expression of Skeletal Muscle in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Park ST, Kim K, Yoon JH, Lee S. Effect of Exercise on GLUT4 Expression of Skeletal Muscle in Streptozotocin-Induced Diabetic Rats. *JEPonline* 2011;14(4):113-122. The purpose of this study was to use the immunofluorescence method to identify whether exercise training increases GLUT4 expression in the cell membrane of skeletal muscle of streptozotocin (STZ)-induced diabetic rats. Sprague-Dawley rats were randomly divided into four groups: control (CON); exercise (EX); diabetes mellitus (DM); and diabetes mellitus with exercise (DM+EX). The treadmill was used for exercise with moderate intensity for 30 min a day, 5 days a week for 6 weeks. We observed that subcellular distribution of GLUT4 determined by immunofluorescence microscopy is entirely located in the cell membrane of muscle fibers. In the DM group, minimal staining can be seen at the cell membrane, but the CON and EX groups show much more staining at the cell membrane. The DM+EX group showed more staining than the DM group. In addition, the results showed that the expression of the number of nuclei per muscle fiber was significantly decreased in the DM group (13.12 ± 0.89) than in the CON group (19.37 ± 0.56) ($P < 0.05$). Also, exercise alleviated diabetes mellitus-induced decrease of nuclei per muscle fiber to the control level (19.56 ± 1.07) ($P < 0.05$). We identified that exercise training increased the expression of GLUT4 in the cell membrane and myonuclei in skeletal muscle fibers of STZ-induced diabetic rats.

Key Words: Training, Diabetes, Immunofluorescence, Myonuclei

INTRODUCTION

Skeletal muscle plays a major role in maintaining homeostasis of blood glucose. It uses glucose as a major source of energy during contractile activity and provides the major disposal site for glucose metabolism during the postprandial phase. Skeletal muscle transports glucose molecules from the blood vessel to the inside of its cell by facilitated diffusion. This procedure is mainly carried by glucose transporter 4 (GLUT4), the primary isoform in the skeletal muscle (34). GLUT4 is the insulin-responsive glucose transporter. It is also known as the muscle-fat glucose transporter, since it is almost exclusively expressed in these two tissues.

Several biochemical studies have suggested that GLUT4 can translocate to both the sarcolemma and t-tubules in response to muscle contraction (27,29). GLUT4 is essential for insulin and/or contraction stimulated glucose uptake in skeletal muscle (35). Therefore, the content of GLUT4 protein is a primary factor in determining the amount of glucose transport into skeletal muscle (14). Skeletal muscles and adipocytes have unique machinery which is required for the movement of GLUT4 from intracellular pools to the plasma membrane (14,32). Stimulation of glucose transport is a major action of insulin and occurs in the insulin target tissues, muscle and fat, by a process involving translocation of the insulin-responsive glucose transporter GLUT4 to the plasma membrane (30), and accounts for a significant part of the insulin activity in the peripheral tissues for the maintenance of glucose homeostasis.

Studies on subcellular fractionation and immunocytochemistry indicate that GLUT4 is absent from the plasma membrane under basal conditions whereas about 40% of the total GLUT4 is present at the cell surface after insulin stimulation (22,31). In addition to insulin, muscle contractions are also important stimuli for GLUT4 expression and translocation from the intracellular storage compartment to the plasma membrane in the skeletal muscle (19,27). The effects of the insulin and contraction stimulated GLUT4 expression and translocation are additive (17). The level of GLUT4 in skeletal muscle increases in response to exercise training (4,16,17), decreases with detraining (25) and may contribute to the changes in insulin action observed with interventions. More specifically, exercise training increases GLUT4 protein expression and translocation to plasma membrane in both lean and obese rats (6,7,10,28).

Insulin resistance, that is the inability of insulin to properly cause glucose uptake into skeletal muscle, is frequently present in obese people and during the development of type 2 diabetes mellitus (T2DM) (33). The T2DM results from an impairment of insulin action. One of the most significant abnormalities in insulin signaling is the decrease of insulin-dependent glucose disposal followed by an increase in hepatic glucose production (20). The exercise-stimulation increases in the translocation of GLUT4 to the skeletal muscle cell membrane occur in people who are healthy and in patients with T2DM (14). So, we tried to use exercise training as an intervention for increasing GLUT4 protein expression in the skeletal muscle of streptozotocin-induced diabetic rats. Immunofluorescence is the labeling of antibodies or antigens with fluorescent dyes. This technique is often used to visualize the subcellular distribution of interest. Therefore, utilization of immunofluorescence staining technique can be very beneficial for investigating the effect of exercise on the distribution of GLUT4 protein in skeletal muscle of diabetic rats. The purpose of this study was to employ the immunofluorescence method to identify whether exercise training increases GLUT4 expression in the cell membrane of skeletal muscle of streptozotocin (STZ)-induced diabetic rats.

METHODS

Animals and Experimental Design

Twenty four 8-week old male Sprague-Dawley rats (body weight 220 ± 34.8 g) were housed individually in a temperature controlled room ($20 \pm 2^\circ\text{C}$) with a 12-hr light/12-hr dark cycle. The experimental procedures were approved by the University's Animal Care and Use Committee. Food and water were made available *ad libitum*. The animals were randomly divided into 4 groups (n=6 in each group): the control group (CON); the exercise group (EX); the diabetes mellitus group (DM); and the diabetes mellitus with exercise group (DM+EX). Diabetes mellitus was induced by intraperitoneal injection of 50 mg/kg streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 10 mM citrate buffer (pH 4.5). The control group and the exercise group were injected with citrate buffer alone. The blood glucose level from tail vein was tested with a glucometer (ACCU-CHEK, Roche Diagnostics, Germany) 48 hrs after injection of STZ to verify the induction of diabetes mellitus if blood glucose level exceeded 300 mg/dl.

Treadmill Exercise Protocols

After verification of diabetes mellitus, the exercise group (EX) and the diabetes mellitus with exercise group (DM+EX) were familiarized with the rat treadmill for a week (0% grade, 5 m/min, 10 min/day). Then, they underwent an exercise training program of moderate intensity for 6 weeks (0% grade, 15 m/min velocity, 30 min/day, 5 days/week). The intensity of exercise was based on a previous study (1).

Muscle Preparation

All rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg) for muscle sampling 48 hrs after last exercise session, and the soleus muscles were dissected out. Muscle samples were rapidly frozen into liquid nitrogen and stored at -80°C and all rats were then euthanized by removal of cardia.

Immunofluorescence staining for GLUT4

Muscle samples were embedded and frozen at -20°C . Sections (20- μm thickness) were cut on a cryostat and mounted on positively charged slides. For immunofluorescence staining, sections were fixed with 4% paraformaldehyde, 4% sucrose in PBS at room temperature for 40 min, permeabilized with 0.5% nonidet P-40 in PBS, and blocked with 2.5% horse serum and 2.5% bovine serum albumin for 4 hr at room temperature. Sections were incubated with anti-GLUT4 antibody (Calbiochem, Cat. No: 400064, Germany), then incubated with fluorescein-goat anti-mouse (Molecular probes, Eugene, OR) or rhodamine-goat anti-rabbit secondary antibodies (Molecular probes) in 2.5% horse serum and 2.5% bovine serum albumin for 1 hr at room temperature and cover-slipped with gelatin mount medium.

Image Analysis

We included control sections treated with secondary antibody alone, which usually did not have any visible images. In cases when the nonspecific signals were high, all the data from those experiments were not further analyzed. Sections were viewed with a fluorescence microscope (Nikon, Japan) and the images were captured by using Nikon camera. The software Adobe was used to acquire images from the digital camera, and the software Adobe Photoshop (version 5.5) was used to process images. To all the sections from the individual experiments, the brightness and contrast of all color images were adjusted essentially to the equal extent when necessary, and the merged images were produced by using layer blending mode options of the Adobe Photoshop program (version 5.5). A fluorescence microscope was used to detect the GLUT4 and myonuclei (Figure 1). Images of the sections were obtained with a digital camera connected to the microscope and were stored on a

personal computer. In each section, 3 different areas contained about 100~120 fibers, and 2,000~2,400 myonuclei, then chosen randomly for calculating number of myonuclei. The average number of myonuclei per muscle fiber was calculated using visual counting technique. The total area observed was digitized on a computer attached to the fluorescence microscope (Nikon, Japan). The same magnification and exposure time were used for all samples.

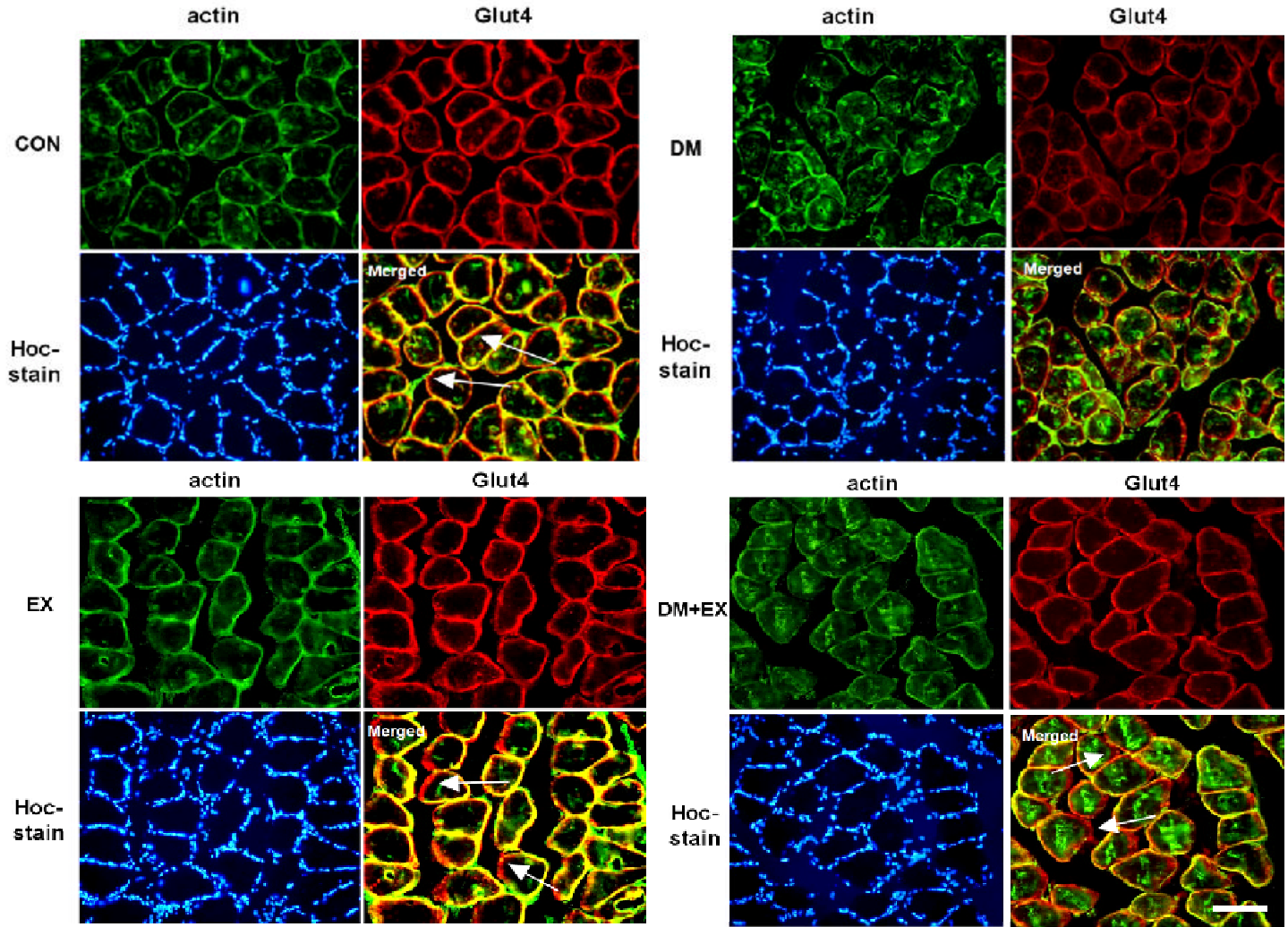


Figure 1. Immunofluorescence staining of GLUT4 in cell membrane of muscle fibers. Merged bands, overlapped with actin, hoechst-stained nuclei and GLUT4 protein, indicate GLUT4 protein expression in beneath cell membrane of muscle fibers (white arrow). Scale bar represents 25 μm (original magnification, $\times 400$).

The Effect of Exercise on the Number of Myonuclei

The number of myonuclei per muscle fiber in the control group was $19.37 \pm 0.56/\text{fiber}$, $25.62 \pm 1.52/\text{fiber}$ in the exercise group, $13.12 \pm 0.89/\text{fiber}$ in the diabetes mellitus group, and 19.56 ± 1.07 in the diabetes mellitus and exercise group (Figure 2). The results show that the expression of number of nuclei per muscle fiber was significantly decreased in the diabetes mellitus group than in the control group ($P < 0.05$). Also, exercise alleviated the diabetes mellitus induced decrease of nuclei per muscle fiber to the control level ($P < 0.05$), while the myonuclear domain was significantly smaller in the exercise group since there was no significant difference in fiber size.

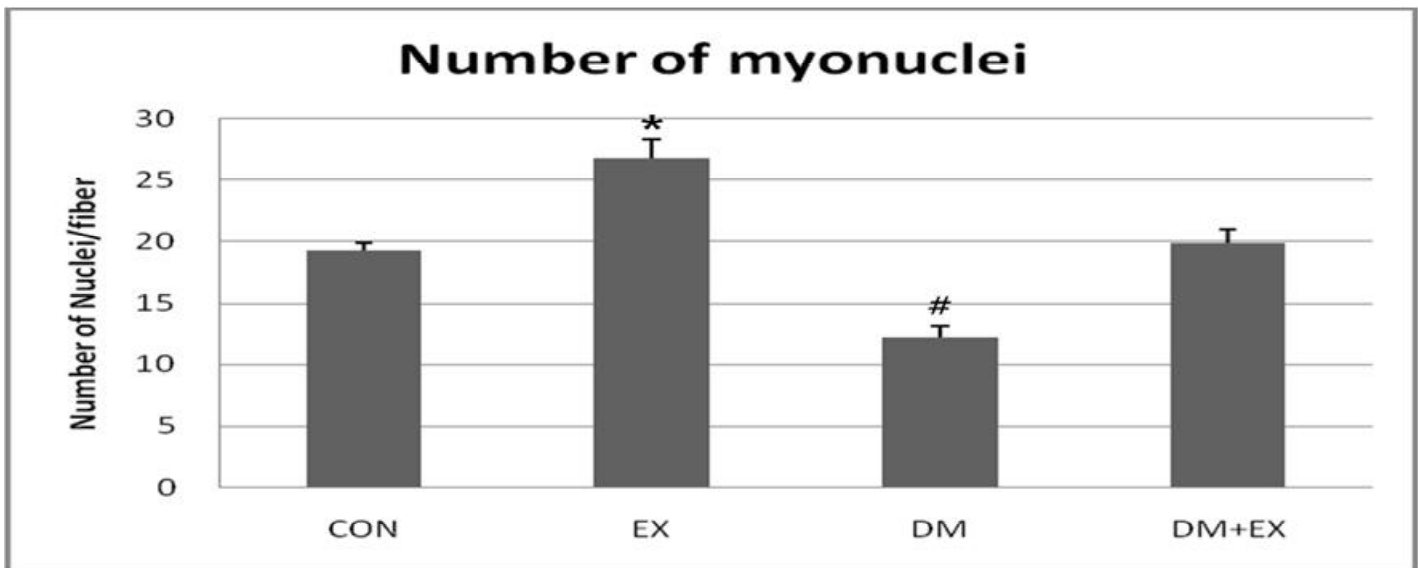


Figure 2. The effect of exercise on the number of myonuclei in the diabetes mellitus rats. CON: Control group, EX: Exercise group, DM: Diabetes mellitus group, DM+EX: Diabetes mellitus with exercise group. *P<0.05 compared to the control group. #P<0.05 compared to the diabetes mellitus with exercise group. Values are represented as the mean \pm S.E.M.

Statistical Analysis

The results are presented as the mean \pm standard error of the mean (S.E.M.). The data were analyzed using SPSS by the one-way analysis of variance (ANOVA) followed by Duncan's *post-hoc* test. The differences were considered significant at P<0.05.

RESULTS

The Effect of Exercise Training on GLUT4 Expressions

We have identified the localization of GLUT4 in the skeletal muscle fiber of STZ-induced diabetic rats using the immunofluorescence staining, and we have also observed that there are distinct differences in thickness of staining owing to treatments of STZ-induced diabetes and/or exercise training. Subcellular distribution of GLUT4 protein determined by immunofluorescence microscopy is entirely located in the cell membrane (Figure 1). Merged bands, overlapped with actin, hoechst-stained nuclei and GLUT4 indicate GLUT4 protein expression in beneath cell membrane of muscle fibers. Remarkably, there is little staining in the diabetes mellitus group (DM), whereas the control group show thick staining. Furthermore, the diabetes mellitus with exercise group (DM+EX) showed more staining than the diabetes mellitus group (DM).

DISCUSSION

Ploug and colleagues (1998) found that GLUT4 in basal fibers is distributed along all the muscle fibers, and is present both at the surface (68% of total GLUT4) and in the core (32% of total GLUT4) of the fibers. They also found that the nuclei are displaced by and aligned with the blood vessels that course along the fiber surface, thereby placing a large fraction of GLUT4 close to the source of glucose and obviating the need for diffusion over long distances. It is well documented that in the basal state, GLUT4 cycles slowly between the plasma membrane and one or more intracellular compartments, with the vast majority of the transporter residing in vesicular compartments within the cell interior (22,27). Insulin stimulated accumulation of GLUT4 protein at the cell surface can be

caused by 10 to 20 fold increase in the rate of exocytosis with a smaller decrease (2 to 3 fold) in the rate of GLUT4 endocytosis (11,15,23). Insulin stimulates glucose transport through GLUT4 translocation from intracellular storage pool to plasma membrane. Insulin resistance caused by insulin deficiency or abnormal insulin signaling results in a decrease in GLUT4 expression, translocation and, then, causes hyperglycemia and diabetes (2,7,12,13). Muscle contraction has also been shown to increase GLUT4 content in the cell membrane (11,22). Many studies suggest that exercise training cause GLUT4 protein expression and translocation to plasma membrane by distinct mechanism from insulin signaling (9,23). In this study, we used only the soleus muscle, generally known as a type I fiber. Most studies on rat skeletal muscle have indicated that more GLUT4 is present in the type I fibers compared with the type II fibers (5,15,23).

The present study follows a study (26) which was undertaken to quantitatively determine whether exercise training increases GLUT4 protein expression in the skeletal muscle of streptozotocin-induced diabetes rats. In the previous study, total content of muscle GLUT4 protein is not affected by type 2 diabetes, whereas GLUT4 mRNA content is reported, variously, to be unaffected or increased (6). However, we found that GLUT4 protein expression of diabetes control rats was lowered more than that of normal control rats, but exercise training reversed diabetes-induced decrease of GLUT4 protein expression (Figure 3) (26).

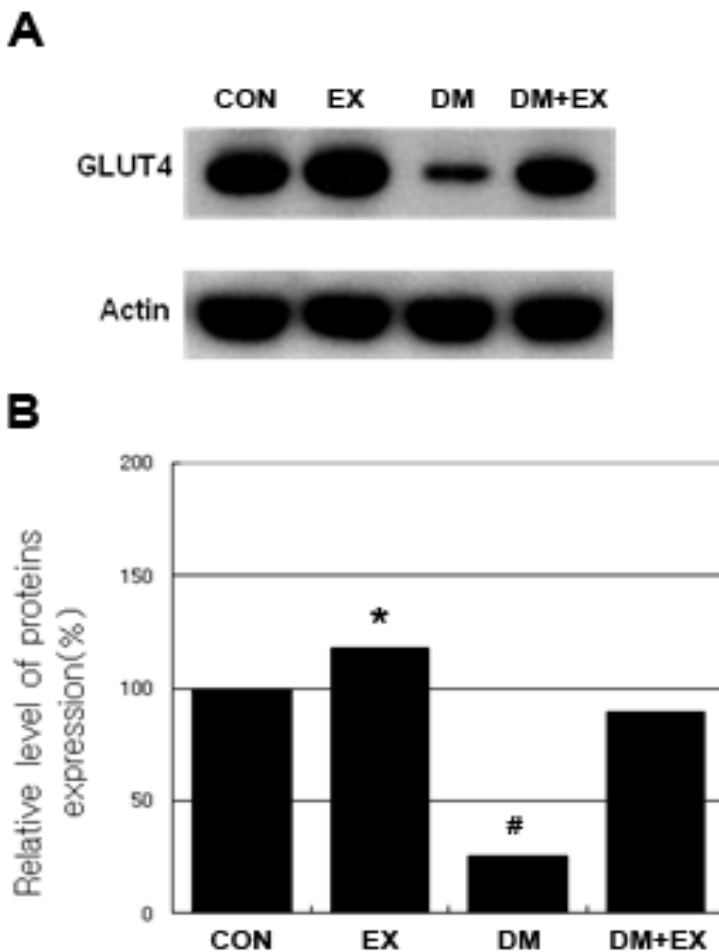


Figure 3. Expression of GLUT4 protein. (A) Western blotting on GLUT4 protein expression in soleus muscle. (B) Quantitative analysis on GLUT4 protein expression in soleus muscle. CON: Control group, EX: Exercise group, DM: Diabetes mellitus group, DM+EX: Diabetes mellitus with exercise group. * $P < 0.05$ compared to the control group. # $P < 0.05$ compared to the diabetes mellitus with exercise group. Values are represented as the mean \pm S.E.M. This figure is a part of the paper which has been previously published (26).

Our study is in agreement with other studies (2,6,11,13,18) that indicate exercise training increased GLUT4 protein expression in insulin deficiency and insulin resistance subjects. These studies also indicated that muscle contraction through physical activity directly modulates GLUT4 expression in the plasma membrane of muscle cell, different from insulin action. Moreover, while the same studies showed the effects of exercise training on GLUT4 protein expression in the cell membrane of skeletal muscle, there has been only a few studies providing visual evidence on this issue.

The number of myonuclei per muscle fiber was significantly increased (3) or not changed by exercise training (8,24) in previous studies. In present study, the number of myonuclei per muscle fiber was significantly higher in the EX group ($P < 0.05$) (Figure 2). We were not able to use a large number of fibers for the calculation of average number of myonuclei per muscle fiber. However, Mackey and colleagues (21) showed that 25 type I and 25 type II fibers are sufficient to estimate the mean number of myonuclei per fiber.

The myonuclear domain should be smaller in the EX group since there was no visible difference in fiber size between CON and EX groups (see Figure 2). By using the immunofluorescence technique, the present study determined that 6 weeks of exercise training increased GLUT4 expression in the cell membrane of the skeletal muscle fibers of STZ-induced diabetes rats. GLUT4 expression in the muscle cell membrane of STZ-induced diabetes rats was significantly lower than that of normal control rats. The exercise training program was able to inhibit diabetes-induced decrease of GLUT4 expression. A limitation of the study is that there were no data about the effects of exercise on markers of insulin sensitivity (i.e., plasma glucose, insulin, and HbA1c in particular) which would strengthen the conclusion that increasing GLUT4 content would benefit diabetes.

CONCLUSIONS

This study identified expressions of GLUT4 in skeletal muscle fibers, using the immunofluorescence method. The exercise training increased the number of nuclei and expression of GLUT4 in the cell membrane in skeletal muscle of STZ-induced diabetes rats.

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