Original Article

Treadmill Training Modifies KIF5B Motor Protein in the STZ-induced Diabetic Rat Spinal Cord and Sciatic Nerve

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Abstract

Background: Previous research has demonstrated diabetic-induced axonal transport deficits. However, the mechanism of axonal transport impairment induced by diabetes is poorly understood. Kinesin motor proteins have been shown to transport various cargos along highly polarized neurons. In the present study, we investigated the effect of regular treadmill exercise on KIF5B and Sunday Driver (SYD) mRNA levels in sensory and motor parts of spinal cord and KIF5B content in sciatic nerves of streptozotocin (STZ)-induced diabetic rats.

Methods: Forty male Wistar rats were divided into four groups: (1) diabetic trained (DT: n = 10); (2) Non-trained diabetic (NTD: n = 10); (3) normal control (NC: n = 10), and (4) normal trained (NT: n = 10). Two weeks after STZ injection (45 mg/kg, i.p.), the rats were subjected to treadmill exercise for 5 days a week over 6 weeks. We determined mRNA levels and protein content by Real time- PCR and ELISA.

Results: Exercise training decreased blood glucose levels in the DT rats. Diabetes increased the KIF5B and SYD mRNA in both sensory and motor parts and KIF5B content in sciatic nerves in the NTD. Moreover, exercise training modulated the KIF5B and SYD mRNA and KIF5B content to normal levels in the DT. Exercise training in NT rats increased KIF5B and SYD mRNA in sensory and motor parts and KIF5B content in sciatic nerves.

Conclusions: Our results suggest that diabetes seems to change spinal cord KIF5B and SYD mRNA and sciatic nerves KIF5B content and exercise training modifies it, which may be attributable to the training-induced decreased hyperglycemia.

Keywords: Diabetes, exercise, kinesin, sunday driver

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Introduction

Among the most prevalent complication of diabetes, neuropathy develops as an irreversible complication in more than half of with diabetes type 1 or 2.[1] Diabetes is associated with slowing of motor nerve conduction velocity and reduced muscle contractile properties.[2,3] In contrast, on the sensory side, there is not only slowing of sensory nerve conduction velocity but also atrophy of perikarya and axons associated with down-regulation of structural protein synthesis and loss of terminal epidermal axons.[4,5]

Several studies have demonstrated that axonal transport deficits in many neurodegenerative diseases might be due to alterations of molecular motor proteins that carry cargoes, structural and regulatory microtubules protein that serve as rail roads, adaptors and scaffold proteins that regulate cargoes attachment to motor proteins, and metabolic modification that disrupt energy supply of motor proteins.[6-12] Among different regulators of axonal motor proteins, SYD as a scaffold protein can interacts with kinesin and mediates the axonal transport of at least three classes of vesicles.[11,12] It was shown that SYD interacts directly with the tail domain of kinesin heavy chain and activates it for microtubule-based transport.[13] Among the 45 kinesin motor proteins that are involved in axonal and intracellular transport, in neuronal cells KIF5B transport synaptic vesicle precursors, membrane organelles, and mitochondria.[14,15] On the other hand, impairment of anterograde axonal transport of some nerotrophic factors and synaptic proteins is demonstrated in neuronal cells of diabetic rats.[16,17] Also, indirect evidence shows that axonal transport of mitochondria may be decreased in diabetic neuropathy.[18]

Previous studies have shown that treadmill exercise training can improve peripheral nervous tissue regeneration in non-diabetic rats and mice after nerve injury and in diabetic rats, improves neuropathic pain and increases axonal regeneration after sciatic nerve transection.[19–24] Exercise training is an interesting model with which increase activation of sensory and motor neurons, axonal transport of proteins, and synaptic remodeling.[25–28] We have previously demonstrated that the amount of CGRP anterogradely transported along axons by fast transport is increased in sciatic motoneurons of exercise-trained rats.[26] These studies have proved that exercise training increases the quantity of axonal proteins and axonal transport, but the effects of exercise training on motor proteins that transport these neurotrophic factors are not elucidated

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yet. Moreover, to our knowledge, no studies have been performed to analyze the effect of diabetes on KIF5B motor proteins expression in the sensory and motor neurons. In the present study, we investigated KIF5B changes in spinal cord and sciatic nerve following the induction of diabetes and treadmill training.

Materials and Methods

Animal characteristics

Experiments were performed on 300 to 320 g male Wistar rats (Razi institute Animal Center, Karaj, Iran). The rats were housed at a constant room temperature of 22 ± 2°C under a 12-h light/dark cycle (lights on at 6:00 AM), with access to food and water ad libitum in the Animal Center of Tarbiat Modares University. The experimental protocols to perform this study were approved by the Ethics Committee on the use of animals of Tarbiat Modares University, Tehran, Iran. All efforts were made to minimize discomfort of the animals and reduce the number of experimental animals. All procedures conformed to the ethical guidelines for the care and use of laboratory animals, published by the National Institutes of Health.

Groups and design

The rats were randomly assigned into 4 groups: (1) diabetic trained (DT), (2) diabetic control (DC), (3) healthy trained (HT), and (4) healthy control (HC). Two weeks after inducing diabetes, 6 weeks of endurance training protocol ensued. For the purpose of familiarization, rats in all groups were adapted to the treadmill by running for 5 days. This protocol was designed as once a day for 10 min/session at a speed of 10 m/min at a slope of 0 degree.

Induction of diabetes

Following an overnight fast, diabetes was induced in the hyperglycemic groups by a single intraperitoneal (i.p.) injection of STZ (45mg/kg; Sigma, St. Louis, MO).[29,30] STZ solution was prepared freshly by dissolving it in 0.5 mol/L citrate buffer (pH 4.0). Control animals received an equivalent volume of citrate buffered solution. As the dose of STZ used and the weight of the rats before STZ treatment are two major factors in causing diabetic complications but not profoundly ill in diabetic rats, in this study a lower dose of STZ and proportionate to rat's weight was used to reduce the severity of illness and the side effects to an acceptable level.[30] Diabetic condition was assessed by serial measurement of non-fasting tail vein blood glucose level using an Accu-Chek Compact Plus blood glucose meter (Roche Diagnostics K.K., Tokyo, Japan) up to 8 weeks after STZ injection, and diabetic rats with constant hyperglycemia (blood glucose levels of more than 350 mg/dL) were included in the study. The body weight and general health was monitored and all animals showed suitable behavior during the entire period of the study.

Exercise training protocol

Rats in training groups were subjected to moderate-intensity treadmill exercise 5 days a week for 6 weeks.[31] The speed and duration of the treadmill exercise gradually increased from 10 m/min for 10 min (grade 0%) in the 1st week to 10 m/min for 20 min (grade 0%) in the 2nd week, 14–15 m/min for 20 min (grade 0%) in the 3rd week, 14–15m/min for 30 min (grade 0%) in the 4th week, and 17–18 m/min for 30 min (grade 0%) from the 5th week. The exercise protocol included 2 to 3 minutes of warm-up and 3 minutes of cool-down. Most animals ran for the majority of the exercise period without any encouragement. The animals did not appear excessively fatigued, which is an important consideration, as exhausting exercise training may alter the levels of lactic acid and cause fatigue that may increase diabetic complications.[32]

Tissue preparation

Twenty four hours after the last training session, animals were anesthetized with a mixture of Ketamine (75mg/kg-1) and Xylazine (5 mg/kg-1) administered intraperitoneally. The lumbar enlargement (L4–L6) was isolated and sensory and motor (or frontal and dorsal) parts were separated using the central canal as a reference and tissues were stored at -70°C until analyzed.[33,34]

Analysis of gene expression by Real-Time PCR

Motor and sensory neuron tissues were transferred to 1 mL lysis reagent (Qiazol; Qiagen) and were homogenized (Tissue-Tearor; Biospec, Bartlesville, OK). RNA extraction was performed identically for both tissues using a purification kit (RNeasy Mini Kit; Qiagen) in accordance with the manufacturer's instructions. Motor and sensory parts mRNA was pooled by successively adding ethanol-precipitated RNA (RNeasy; Qiagen). Concentrations of RNA were determined by UV spectrophotometry (Eppendorff, Germany). Total cDNA synthesis was performed using ABI PRISM cDNA arhive kit in 0.2 mL microfuge tubes. The reaction mixture of 20 µL contained 0.2 µg total RNA, 10×RT buffer, 25 × dNTP mixture, 10 × random primers, MultiScribe RT (50 U/µL) and RNase free water. The cDNA synthesis reactions were carried out for 20 minutes at 42°C, followed by denaturation for 5 minutes at 95°C and 5 minutes at 4°C.

Real-time PCR assays were performed in 48-well plates in ABI 7300 real-time PCR instrument (Applied Biosystems). The primers and probes were purchased from Applied Biosystems, Foster City, California, USA. The forward and reverse primers for KIF5B gene were 5′-GATGTAAAGCAACCGGAGGGG and 5′-TGTTGGGAGATACGAAGCTGG. The forward and reverse primers for SYD gene were 5′-CCAGCTACCAGTGTCCAAACGAT and 5′-CTTTGTGACACTGCCATAGTCCC. The forward and reverse primers for GAPDH (reference gene) were 5′-GACATGCCGCCTGGAGAAAC and 5′-AGCCCAGGATGCCCTTTAGT.

The TaqMan reaction mixture of 20 µL contained 25 ng of total RNA-derived cDNAs, 200 nM each of the forward primer, reverse primer, and TaqMan probe for target gene and endogenous control (GAPDH) and 12.5 µL of Taqman 2 × Universal PCR Master Mix (Applied Biosystems) and the volume was made up with RNAse free water. The following thermal cycling profile was used (40 cycles): 50°C for 2 min, 95°C for 10 min, 95°C for 15 sec and 60°C for 1 min. The quality of the real-time PCR reactions was confirmed by melting curve analyses. Efficiency was determined for each gene using a standard curve (logarithmic dilution series of cDNA from the testes). For each sample, the reference gene (GAPDH) and target gene were amplified in the same run. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control GAPDH in the same samples (ΔCT = CTTarget – CT GAPDH). It was further normalized with the control (ΔΔCT = ΔCT–CTControl). The fold change in expression was then obtained as $2^{-\Delta\Delta CT}$.[35]

KIF5B assay

Levels of KIF5B in the homogenized (1:10 in 10MmPBS, pH 7.4 at 4ºC) and centrifuged (20000 rpm/45 min) sciatic samples were determined using Rat Kinesin Family Member 5B ELISA kit (RayBio®, Hölzel Diagnostika, Köln, Germany) according to the manufacturer's instructions.

Statistical analysis

All data were analyzed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Data are presented as mean ± SEM. Descriptive 2-way analysis of variance (ANOVA) was used in different groups. If analysis were significant, the differences between groups were estimated using Tukey's HSD *post hoc* test. Significance was defined as $P < 0.05$.

Results

3.1. Effect of diabetes and exercise on body weight and blood glucose levels

All rats in training groups completed the 6-week endurance training program. Compared with HT and HC groups, the DT and DC groups developed significantly reduced body weight ($P = 0.0001$ and $P = 0.001$, respectively). Also, the weight of DT group than in DC group was significantly lower ($P = 0.04$). However, the weight of HT group was lower than the HC group, but this difference was not statistically significant ($P = 0.1$).

The change in blood glucose levels is shown in Figure 1. Diabetic rats developed hyperglycemia within 2 days after STZ injection. Before training, blood glucose concentration was significantly higher in diabetic groups compared with normal groups ($P = 0.0001$), and was still statistically significant after 6 weeks of endurance training ($P = 0.0001$). Also, after training period, blood glucose concentration was significantly lower in DT compared with DC ($P = 0.0001$). These results indicate that the STZ-induced diabetic groups (i.e., DT and NTD) presented higher blood glucose levels than the normoglycemic rats and endurance training exercise can decrease blood glucose levels in STZ-induced diabetic trained rats.

3.2. Diabetes increases KIF5B and SYD mRNA levels and exercise can modify it in spinal cord

Real Time-PCR analysis showed that compared with normal control, KIF5B and SYD mRNA levels were markedly elevated 4.5- and 2.5-fold in motor parts and 2.7- and 3.9-fold in sensory parts of DC rats ($P = 0.0001$) and endurance training could significantly modulate them in sensory and motor parts of DT rats ($P = 0.0001$). Compared with normal control, KIF5B and SYD mRNA levels were markedly elevated 3.1- and 4.1-fold in motor parts and 3.4- and 2-fold in sensory parts of HT rats ($P = 0.0001$) (Figures 2 – 5). Taken together, these results indicated that diabetes and exercise can affect axonal motor protein.

3.3. Diabetes increases KIF5B content and exercise can modify it in sciatic nerve

Results showed that diabetes leads to significant increase in KIF5B content of sciatic nerve in DC rats ($P = 0.001$) Moreover, there was no significant difference between KIF5B content of sciatic nerve in DT and HC rats ($P = 0.55$). Compared with HC group, endurance training significantly increased KIF5B content of sciatic nerve in HT rats ($P = 0.001$, Figure 6).

Discussion

The current study tested the hypothesis that diabetes and exercise can affect axonal motor protein that is involved in neuronal transport of synaptic proteins, mRNA and mitochondria in sensory and motor neurons of spinal cord of STZ-induced diabetic rats. The results of present study are the first to demonstrate that diabetes up-regulates KIF5B and SYD mRNA at lumbar sensory and motor neurons and protein levels at sciatic nerves of STZ-induced diabetic rats. Moreover, exercise training modulated both KIF5B and SYD mRNA in lumbar sensory and motor neurons and KIF5B levels in sciatic nerves of diabetic trained rats and rendered them close to normal levels. These results indicated that lumbar sensory and motor neurons' KIF5B and SYD mRNA and sciatic nerves' KIF5B levels in diabetic state could be changed and exercise treadmill training can modify it. Exercise training also increased lumbar sensory and motor neurons KIF5B and SYD mRNA and sciatic nerves' KIF5B levels of healthy trained rats.

Maintenance and function of neurons are dependent on the intracellular transport of organelles along the axons. Basically, having a good synaptic function and mitochondria for local energy requirements in neurons are dedicated to molecular motor proteins that anterogradely transport cargoes.[6] Neurodegenerative diseases are characterized by significant defects in synaptic function and "dying-back" degeneration of axons.[7,8] Accordingly, alterations in fast axonal transport have been documented in Alzheimer's, Parkinson's and Huntington's diseases and amyotrophic lateral sclerosis.[9,36–39] These diseases are associated with mutations/dysfunctions in molecular motor proteins or alterations in the activity of specific protein kinases that have been implicated in regulation of these motor proteins' function.[6–8] In the present study, we showed that diabetes up-regulates SYD mRNA at lumbar sensory and motor neurons of STZ-induced diabetic rats and exercise treadmill training can modify it. It was demonstrated that SYD's interaction with kinesin heavy chain can mediate kinesin transport along microtubules and determine the destination of kinesin-1 and its adaptor.[13] Moreover, vesicular structures in axons containing SYD are transported in both the anterograde and retrograde axonal transport pathways and interact with kinesin-1 and the dynactin complex. But in conditions of nerve injury, SYD binds either kinesin or dynactin, but not both simultaneously.[40] These studies suggests that upon encountering an injury, SYD switch direction of axonal transport to the retrograde pathway. Generally, it can be noticed that molecular motor proteins and their regulator seem to be the target of neurodegenerative diseases. For example, mutation in KIF5A results in a hereditary form of spastic paraplegia, a disease characterized by progressive dysfunction and degeneration of upper motor neurons.[41] Moreover, kinesin mutations are causative of Charcot-Marie-Tooth (CMT) disease.[42] In agreement with these studies, our results show that diabetic state changed neuronal KIF5B motor protein and endurance exercise training modified this change.

Our results show that diabetes as a neurodegenerative disease and endurance exercise training as a neuroprotective strategy can increase KIF5B levels.[1,26,43] In agreement with our results, Maeda, et al. (1996) reported increased GAP-43 mRNA in DRGs of diabetic rats compared to control rats.[44] Among the other neurotrophic factors that are transported by kinesin motor proteins, mRNA and protein content of BDNF have been up regulated in the hip-

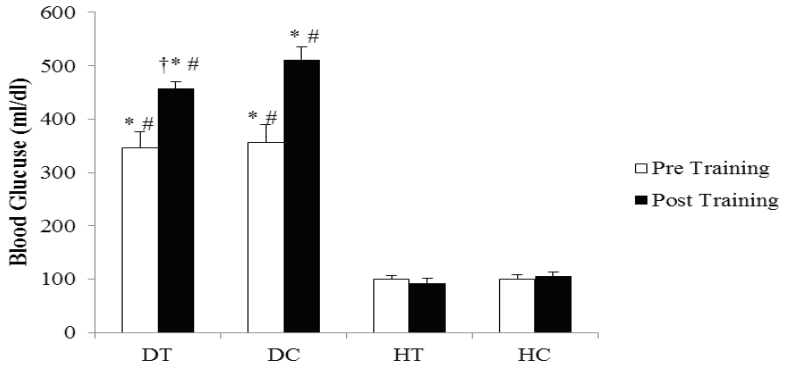

Figure 1. Blood glucose levels in different groups, where DT = diabetic trained, DC = diabetic control, HT= healthy trained and HC = healthy control. The values are presented as mean ± SEM for 7 rats per group. Symbols (*, # and †) indicate significant differences with HC (P = 0.0001), HT (P = 0.0001) and DC (P = 0.0001) respectively.

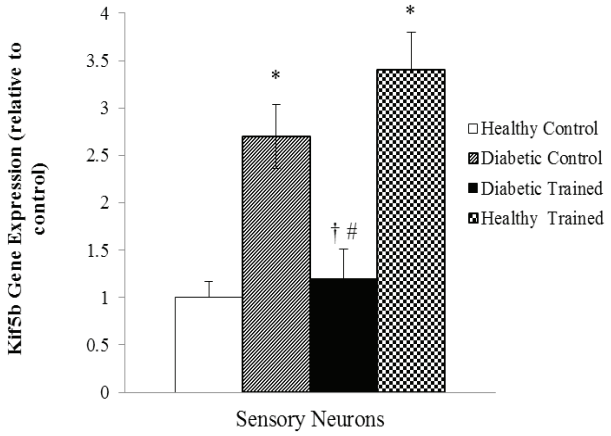

Figure 2. Representative graph showing Real-Time amplification of KIF5B mRNA in sensory parts. The values are presented as mean ± SEM for 4 rats per group. Symbols (*, # and †) indicate significant differences with HC (P = 0.0001), HT (P = 0.0001) and DC (P = 0.0001) respectively. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control GAPDH in the same samples (ΔCT = CTTarget – CT GAPDH). It was further normalized with the control (ΔΔCT = ΔCT – CT-Control). The fold change in expression was then obtained ($2^{-\Delta\Delta CT}$). The graph was plotted using log $2^{-\Delta\Delta CT}$. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with GAPDH CT value as the internal control and Control CT value as the calibrator.

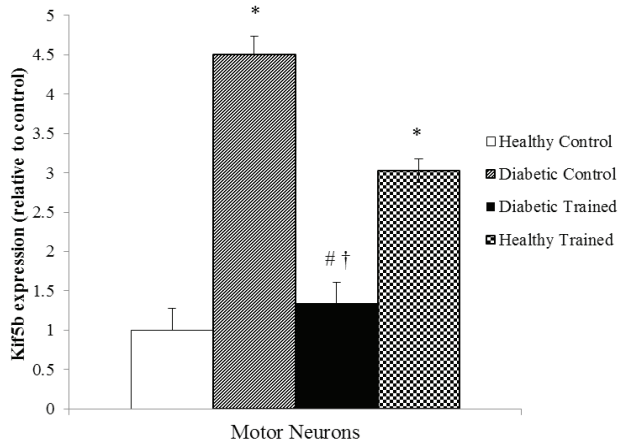

Figure 3. Representative graph showing Real-Time amplification of KIF5B mRNA in motor parts. The values are presented as mean ± SEM for 4 rats per group. Symbols (*, # and †) indicate significant differences with HC (P = 0.0001), HT (P = 0.0001) and DC (P = 0.0001) respectively. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control GAPDH in the same samples (ΔCT = CTTarget – CT GAPDH). It was further normalized with the control (ΔΔCT = ΔCT – CT-Control). The fold change in expression was then obtained ($2^{-\Delta\Delta CT}$). The graph was plotted using log $2^{-\Delta\Delta CT}$. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with GAPDH CT value as the internal control and Control CT value as the calibrator.

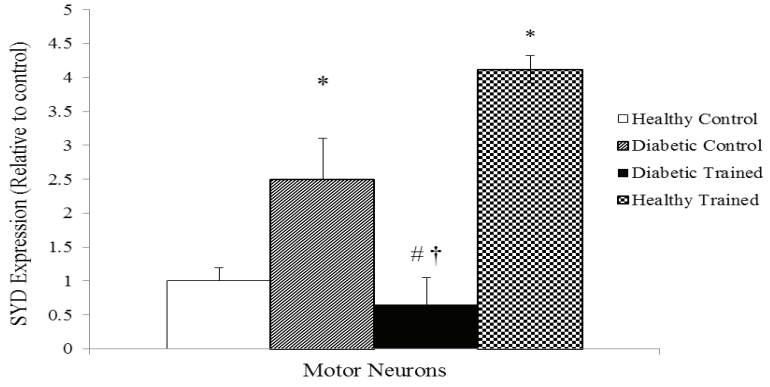

Figure 4. Representative graph showing Real-Time amplification of SYD mRNA in motor parts. The values are presented as mean ± SEM for 4 rats per group. Symbols (*, # and †) indicate significant differences with HC (P = 0.0001), HT (P = 0.0001) and DC (P = 0.0001) respectively. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control GAPDH in the same samples (ΔCT = CTTarget – CT GAPDH). It was further normalized with the control (ΔΔCT = ΔCT – CTControl). The fold change in expression was then obtained ($2^{-\Delta\Delta CT}$). The graph was plotted using log $2^{-\Delta\Delta CT}$. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with GAPDH CT value as the internal control and Control CT value as the calibrator.

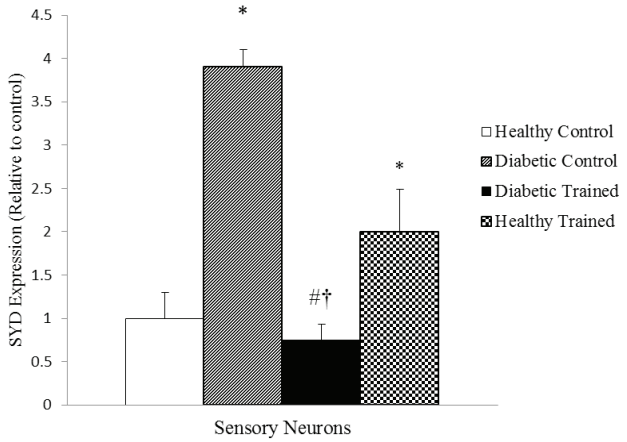

Figure 5. Representative graph showing Real-Time amplification of SYD mRNA in sensory parts. The values are presented as mean ± SEM for 4 rats per group. Symbols (*, # and †) indicate significant differences with HC ($P = 0.0001$), HT ($P = 0.0001$) and DC ($P = 0.0001$) respectively. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control GAPDH in the same samples (ΔCT = CTTarget – CT GAPDH). It was further normalized with the control (ΔΔCT = ΔCT – CT-Control). The fold change in expression was then obtained ($2^{-\Delta\Delta CT}$). The graph was plotted using log $2^{-\Delta\Delta CT}$. The relative ratios of mRNA levels were calculated using the ΔΔCT method normalized with GAPDH CT value as the internal control and Control CT value as the calibrator.

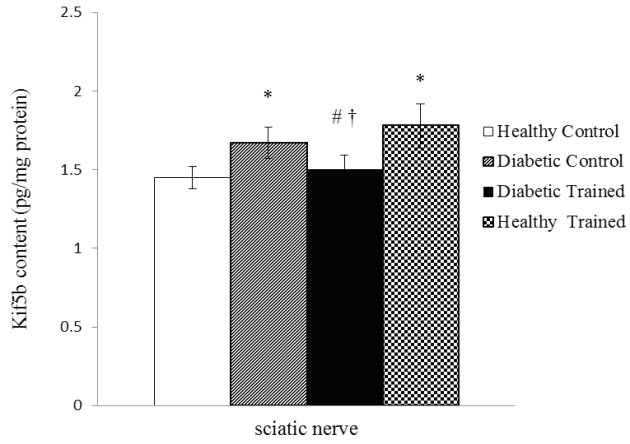

Figure 6. Representative graph showing KIF5B content (pg/mg protein) in sciatic nerve. The values are presented as mean ± SEM for 4 rats per group. Symbols (*, # and †) indicate significant differences with HC ($P = 0.001$), HT ($P = 0.001$) and DC ($P = 0.001$), respectively.

pocampus of STZ induced diabetic rats.[14,15,43] Also, elevated levels of KIF1A motor protein in substantia nigra of rat model of α-synucleinopathy[45] were attributed to the imbalance in protein degradation and synthesis or to axonal transport deficit. Neuropathy in diabetic neurons leads to peripheral nerve degeneration which manifests as persistent neuropathic pain,[46] and our results showed increased lumbar sensory and motor neurons KIF5B and SYD mRNA and sciatic nerves' KIF5B levels in diabetic rats. In the present study, the similarity between KIF5B mRNA and protein content in neuronal cells of non-diabetic and diabetic rats indicates that neurons may respond by an attempt to activate their machinery for regeneration as a compensatory mechanism.

By interacting with kinesin-1, SYD mediates microtubule-based transport of various cargoes in neurons.[11,40] In the present study, we showed that exercise training leads to up-regulation of SYD mRNA in lumbar sensory and motor neurons of non-diabetic rats. This result indicates that exercise training at transcriptional level can positively affect SYD as a key regulator for axonal transport.

Transporting synaptic vesicles is one of the most important roles of these motor proteins.[14,15] Our results show that diabetes might change molecular motor which is involved in synaptic function and exercise can modify this condition. Confirming these data, synaptic degradation of nerves was demonstrated in STZ-induced diabetic rats.[47] Moreover, the activity-dependent regulation of gene expression occurs even in the adult brain and it is believed to play an important role in synaptic plasticity.[48] Increased afferent inputs of a neuron by exercise training or electrical stimuli bring long-term potentiation (LTP) as a form of long-lasting synaptic plasticity adaptation in both CNS and PNS.[49,50] It is demonstrated that synthesis of new mRNA and proteins is essential for the induction of LTP.[51] Moreover, several studies have revealed that the induction of synaptic plasticity depended on the transcription of many genes.[52,53]

Mitochondria are another cargo in neuronal cells that are transported mainly by KIF5B motor protein.[14,15] Mitochondria complex mobility patterns within neurons are characterized by frequent changes in direction. Defects in mitochondrial transport are implicated in the pathogenesis of many neurodegenerative diseases, including Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Charcot-Marie-Tooth disease.[54] Also, mitochondrial dysfunction has been proposed as a major mediator of neurodegeneration in diabetes and indirect evidence showed that axonal transport of mitochondria may be decreased in diabetic neuropathy.[18,55,56] Our results suggest that diabetic state changes sciatic levels of KIF5B motor protein which is involved in axonal transport of mitochondria.

Interestingly, our results show that endurance exercise training can up-regulate expression and protein content of KIF5B motor proteins in sensory and motor neurons and sciatic of non-diabetic rats, respectively. We have previously demonstrated that the amount of anterograde axonal transport of CGRP, that is a KIF5B cargo, is increased in sciatic motoneurons of exercise-trained rats. Also, KIF5B transports SNAP-25 and substance P and chronic exercise increases axonal transport of SNAP-25 in motoneurons of rat and substance P release in the spinal cord of cats.[26,28,57,58]

Although we did not provide direct evidence of the mechanism of hyperglycemia to alter KIF5B expression in this study, accumulated evidence shows that hyperglycemia appears to be an important factor for neurodegeneration in diabetes which has been associated with reduced axonal transport rates in STZ-induced diabetic mice.[32,59] But the present study demonstrated that endurance training exercise significantly decreases blood glucose levels in STZ-induced diabetic trained rats. Axonal transport defects in diabetic mice were attributed to hyperglycemia-induced oxidative stress damages.[32] Chronic hyperglycemia imposes ischemic, hypoxic, oxidative, and apoptotic stress leading to widespread damage to proteins, cells, and tissues.[60,61] Chaperone molecules also play an important role to minimize hyperglycemic stress.[62] Moreover, previous studies have suggested that chaperone molecules

can regulate kinesin-driven axonal transport and completely reverse inhibited kinesin-1 axonal transport in amyotrophic lateral sclerosis.[10,63] In addition, some neuronal degenerative characteristics of diabetes were attributed to defects in chaperone proteins and exercise training demonstrated that up-regulate chaperones in STZ-induced diabetic rat neurons.[64,65] Among the other factors contributing to changes in axonal transport in diabetes, proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β, are up-regulated in the hippocampus of diabetic BB/Wor rats.[66] Studies show that TNF can negatively regulate kinesin activity in L929 cells.[67] Moreover, swimming and treadmill exercises increase HSP- 72 , TNF-α and IL-1β contents in sciatic nerve of CCI rats.[68]

Glycogen synthase kinase 3 (GSK3) and cyclin dependent kinase 5 (CDK5) are other protein kinases that negatively regulate kinesin-mediated transport and their impairment has been reported in sensory neuron of diabetic BB/Wor rats.[7,69] Microtubule-associated proteins (MAPs) are other factors that could be changed in impaired axonal transport.[6] Moreover, excessive tau phosphorylation is known to disrupt kinesin motor proteins trafficking and abnormal phosphorylation of tau was detected in the brain of STZ-induced diabetic rats.[70,71] Evidence has been presented that chronic exercise decreases the levels of phosphorylated tau in the brain of mice model of Alzheimer's disease.[72] Further studies will be needed to determine if similar pathways may be active under diabetic conditions, therefore contributing to the detected changes in the mRNA levels of motor proteins in the sensory and motor neurons.

The results of the present study and other researches in the field of axonal transport impairments in neurodegenerative diseases may be supportive of "cargo and driver theory". This theory explains that in neurodegenerative diseases such as diabetic neuropathy, impaired axonal transport is due to two reasons. First, down-regulation of neurotrophic factors and other proteins which are essential for neuronal survival.[55,73,74] Second, impairment in motor proteins that actively transport these cargoes.[6,7] Another important issue related to this theory refers to the modifying role of exercise. However, there are other related points which should be explained by future studies.

In conclusion, the present study demonstrates that the STZ-induced diabetic rats demonstrate changed KIF5B and SYD mRNA levels in spinal cord sensory and motor neurons and KIF5B sciatic content, and chronic endurance exercise training can modify these changes which may contribute to the mechanisms of decreased hyperglycemia in diabetes. Moreover, exercise training can up-regulate KIF5B and SYD mRNA levels and protein content of KIF5B motor proteins in sensory and motor neurons and sciatic of non-diabetic rats, respectively. Our results suggested a potential role for KIF5B and SYD as a novel treatment of diabetes. It can be concluded that neuronal machinery system at transcriptional and post-transcriptional levels might be affected in diabetic state and exercise can modify it and these results are consistent with the "cargo and driver theory" in neurodegenerative diseases.

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