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Swimming Exercise Induced Reversed Expression of miR-96 and Its Target Gene $Na_V1.3$ in Diabetic Peripheral Neuropathy in Rats

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Abstract

Diabetes is a common metabolic disease which leads to diabetic peripheral neuropathy (DPN). Recently, the role of micro-ribonucleic acid-96 (miR-96) in alleviating neuropathic pain by inhibiting the expression of $Na_V1.3$, an isoform of voltage-gated sodium channels, has been shown. Peripheral nerve injuries result in $Na_V1.3$ elevation. Exercise has beneficial role in diabetes management and peripheral neuropathy. However, the effects of exercise on miR-96 and its target gene $Na_V1.3$ in diabetic rats are unknown. Therefore, the present study investigated the effects of exercise training on the expression of miR-96 and $Na_V1.3$ in diabetic rats. For this purpose, rats were randomly divided into four groups: control, exercise, diabetic and diabetic-exercise groups. Type 2 diabetes was induced by a high-fat diet and the administration of streptozotocin (STZ) (35 mg/kg, i.p.). The exercise groups were subjected to swimming exercise 5 days/week for 10 weeks. At the end of the treatment period, thermal pain threshold, determined through the tail-flick test, and the expression levels of miR-96 and its target gene $Na_V1.3$ were determined by reverse transcription -polymerase chain reaction (RT-PCR) in the sciatic nerve tissues of the rats. Data of the present study indicated that diabetes diminished miR-96 expression levels, but significantly upregulated $Na_V1.3$ expression in the sciatic nerve. On exercise training, miR-96 expression was reversed with concurrent down-regulation of the $Na_V1.3$ expression. This study introduced a new and potential miRNA-dependent mechanism for exercise-induced protective effects against diabetic thermal hyperalgesia.

Key Words: diabetic neuropathy, exercise, miR-96, $Na_V1.3$, rat, sciatic

Introduction

Diabetes is one of the most common metabolic diseases with increasing prevalence worldwide (15, 26). Diabetes induces lesion or dysfunction of the peripheral nervous system and leads to diabetic peripheral neuropathy (DPN) pain, which impairs the quality of life of the diabetic patients (4, 32). This neuropathy is the most common form of diabetic neuropathies (4).

The pathogenesis of diabetic neuropathy remains unclear (16). One possible mechanism is dysregulation of ion channels. In this case, the role of $Na_V1.3$ as an isoform of voltage-gated sodium channels in diabetic neuropathy pain has been documented (35). It has also been shown that inhibition

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of $Na_V1.3$ results in improvement of neuropathy pain (8). This voltage-gated sodium channel, which is encoded by the *Scn3A* gene, is largely expressed in dorsal root ganglion (DRG) neurons in the embryonic period and *Scn3A* gene expression diminishes to undetectable levels postnatally. However, the expression of $Na_V1.3$ increases due to peripheral nerve injuries, which has a pivotal role in the occurrence of neuropathic pain (35).

Micro-ribonucleic acids (miRNAs) are short non-coding RNAs which have regulatory effects on the expression of their target genes through inhibiting protein translation (5, 34). Recently, the roles of altered expression of miRNAs in neuropathic pain are shown (8, 21, 37). According to a study by Chen *et al*., an important miRNA which has been shown to be involved in neuropathic pain is miRNA-96 (miR-96). It was shown that miR-96 improved neuropathic pain by inhibiting the expression of $Na_V1.3$ (8).

In spite of a growing list of effective drugs, DPN remains under-treated (28) and it cannot be completely alleviated by current medications (31). Aerobic and resistance training exercises are effective therapeutic intervention for type 2 diabetes mellitus (T2DM) (7, 13, 22). It has also been shown that regular exercise has positive effects on blood glucose, insulin sensitivity, hypertension and dyslipidemia in diabetic patients (3, 14). Recently, it has also been suggested that exercise training is beneficial for various symptoms related to peripheral neuropathy (12). Because of a possible involvement of miRNAs in DPN (8, 37), we hypothesized that exercise training might positively affect this diabetic complication through modulating miRNAs and their target genes. In this regard, the current study aimed to investigate the effects of exercise training on the expression of miR-96 and $Na_V1.3$ in T2DM rats.

Materials and Methods

Animals

Forty-eight male Wistar rats, weighting 200-250 g, were obtained and kept under standard conditions of 22 ± 2°C and 12:12 light-dark cycle with free access to food and water *ad libitum*. All authors of this study ensured that the animals were cared in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA), and that the use of the animals was reviewed and approved by the animal care review committee at the Tabriz University of Medical Sciences (IR.TBZMED.REC.1393.1077).

Experimental Procedures

Animals were randomly divided into four groups with twelve rats per group. In each group, six rats were used for the behavioral analysis and six for gene expression analysis. The four groups were: control, exercise (swimming training), diabetic and diabetic-exercise groups. After an acclimatization period of one week for adaptation with room and cages, experimental procedures were done over a 10-week period.

For induction of type 2 diabetes, animals were fed with a high-fat diet, consisting 58% fat, 17% carbohydrate and 25% protein, for one month after which animals were switched to a standard diet. At the end of the 4^{th} week, all animals were weighed, which was taken as the initial weight, and rats of the diabetic groups received streptozotocin (STZ) (35 mg/kg) *via* intraperitoneal injection (38). After 72 h, blood glucose levels were measured by a digital glucometer (Gluco Sure, Star, Taiwan), and animals with blood glucose levels exceeding 300 mg/dl were considered as diabetic rats.

Rats in the exercise groups were exposed to a swimming pool of 100 × 60 × 100 cm containing water at 34-36°C. The training protocol consisted of 60 min/session and 5 days/week for 10 weeks. At the end of each training session, rats were thoroughly dried and placed in a moderately warm room for 60 min. In this period, the animals in the "non-exercise" groups remained in the home cages. At the end of the exercise period (10 weeks), tail-flick latency (TFL) and the expression levels of miR-96 and $Na_V1.3$ were measured in all groups.

Tail-Flick Protocol

At the end of the training period, six rats from each group were randomly selected and were exposed to the tail-flick test. Nociceptive threshold was measured by the tail-flick system. In this test, the distal 4 cm of the tail was subjected to nociceptive stimulus with an irradiation intensity of 20 $mWatt/cm^2$ and a 30-second cut-off time to prevent excessive injuries (25). At end of the experiment, TFL period(s) from the radiant heat was recorded.

Sample Collection

At the end of the training period, body weight, which was now taken as the end weight, and fasted blood glucose levels of the remaining six rats of each group were measured. The animals were then anesthetized with ketamine (60 mg/kg) and xylazine

Table 1. Primers used for qPCR analysis.

Gene	Accession number	Primers sequence[a]
$Na_V1.3$	Y00766.1	F: AGGGAAGGATTGACTTGCC R: TGGACCTCTCCTTAGAGTCCA
β-actin	NM_031144.3	F: TACAGCTTCACCACCACAGC R: ATGCCACAGGATTCCATACC
rno-miR-96-5p	MIMAT0000818	UUUGGCACUAGCACAUUUUUGCU
rno-miR-191-5p	MIMAT0000866	CAACGGAAUCCCAAAAGCAGCUG

[a]For mRNAs, sequences were derived from NCBI (www.ncbi.nlm.nih.gov); for miRNAs, sequences were derived from miRBase (www.mirbase.org).

Table 2. Blood glucose levels, bodyweights, and TFL period in the rats.

Group	Fasting blood glucose (mg/dl)	Initial body weight (g)	End body weight (g)	TFL period (seconds; mean ± SD)
Control	108 ± 1.95	242 ± 3.008	308.1 ± 2.36	10.5 ± 1.15
Exercise	107 ± 1.61	240 ± 3.27	316.4 ± 1.797	18.48 ± 1.7*
Diabetic	461 ± 12.7*	276 ± 2.75*	194.8 ± 2.186*	5.5 ± 0.65*
Diabetic-exercise	382 ± 25.97*,#	275.4 ± 3.54*	211.6 ± 2.033*,#	10.88 ± 0.48#

Data are expressed as mean ± SEM. *$P < 0.05$ versus the control group #$P < 0.05$ versus the diabetic group.

(10 mg/kg) and after decapitation, sciatic nerve samples were removed for total RNA extraction for expression assays of miR-96 and $Na_V1.3$ mRNA by real-time PCR.

Real-Time Quantitative PCR (qPCR)

Total RNA was extracted from the sciatic nerve tissues using the RNX-Plus solution kit (Fermentase, Cinagen Co., Tehran, Iran) and miR-amp kit (Parsgenome Co, Tehran, Iran) according to the protocols of the manufacturers. The cDNA synthesis and real-time PCR procedures were performed as previously described (17). The qPCR reactions were performed using primers listed in Table 1. The PCR method was conducted by the use of SYBR Green PCR Master Mix (Fermentas, Cinagen Co., Tehran, Iran) on a real-time PCR machine (Rotor-Gene 3000) (17). The relative quantifications of $Na_V1.3$ and miR-96 expression levels were compared with the internal reference β-actin for $Na_V1.3$ mRNA, and rno-miR-191 for miR-96, and the fold change results were computed using the $2^{-\Delta\Delta Ct}$ method (36).

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) and tukey *post-hoc* test by SPSS 16.0 software. $P < 0.05$ was considered statistically significant.

Results

As shown by Table 2, diabetes induction significantly increased ($P < 0.05$) the blood glucose levels, and exercise could reduce ($P < 0.05$) this variable in the diabetic-exercise group compared to the diabetes group. Moreover, the end bodyweight was significantly decreased ($P < 0.05$) in the diabetic rats compared with rats in the control group. However, swimming training increased ($P < 0.05$) the end bodyweight of the diabetic rats (Table 2).

In the other part of this study, latency time in tail-flick test was used to evaluate thermal pain threshold. Results showed that diabetes significantly diminished ($P < 0.05$) the latency time, and swimming training significantly enhanced ($P < 0.05$) the latency time in the diabetic rats. Furthermore, swimming exercise significantly increased ($P < 0.05$) the latency time in the healthy control rats (Table 2).

Results showed that diabetes non-significantly decreased miR-96 expression (Fig. 1A). Also as

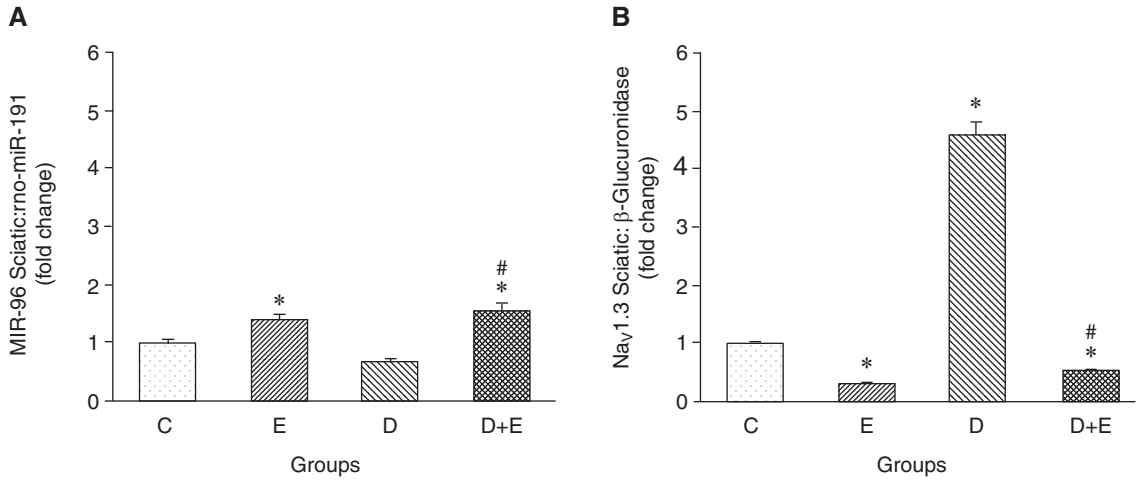

Fig. 1. Sciatic expression levels of miR-96 (A) and $Na_V1.3$ (B) in control and test groups (C: control, E: exercise, D: diabetic, D+E: diabetic-exercise). Data are expressed as mean ± SEM. $^*P < 0.05$ versus C, $^{\#}P < 0.05$ versus D. The data shown were derived from six independent experiments for each group.

shown in this figure, swimming exercise training significantly upregulated ($P < 0.05$) the expression of miR-96 in the exercise groups relative to the control or diabetic rats. $Na_V1.3$ expression in the study groups is shown in Fig. 1B. The data showed that diabetes enhanced ($P < 0.05$) $Na_V1.3$ expression compared to the control group, and exercise training reversed this change, which reached a level just under the control values ($P < 0.05$). Moreover, significantly reduction ($P < 0.05$) in the $Na_V1.3$ expression was observed in healthy rats after the exercise training (Fig. 1B).

Discussion

The role of miRNAs in metabolic disease, for instance diabetes and obesity, has been documented. The noncoding RNAs are considered as therapeutic targets for many diseases such as diabetes and its complications (6). It is also shown that exercise plays a beneficial role in diabetes mellitus (14, 27). The present study examined possible mechanisms of exercise-induced protective effects on diabetic neuropathy pain through alteration in gene regulation. Data of this study revealed that diabetes induced thermal hyperalgesia and exercise training protected rats from diabetic thermal hyperalgesia. Furthermore, beneficial effects of exercise in diabetic neuropathy pain have been shown by previous studies (1, 20, 29). Kluding *et al*. reported that aerobic and resistance exercise significantly improved DPN-related pain in human subjects (20). Also, Rossi *et al*. suggested that swimming training decreased thermal hyperalgesia in STZ-induced diabetic rats (29). Therefore, as suggested by the present and previous works, regular exercise can improve diabetic neuropathy-related thermal hyperalgesia. We also evaluated possible miRNA-related mechanism of this protective effect. It has been recognized that miR-96 regulates neuropathic pain through its inhibitory effect on $Na_V1.3$ (8). Data of this study showed that diabetes non-significantly reduced miR-96 and, thus, significantly elevated $Na_V1.3$ expression. These findings are in agreement with results of a previous study which showed that miR-96 level was diminished in DRG after peripheral nerve injury, and that the $Na_V1.3$ level was upregulated (8). Results of the present study were also consistent with those of Hong *et al*. who found that $Na_V1.3$ expression increased in STZ-induced diabetes and resulted in painful diabetic neuropathy (19). Another previous study proposed that peripheral axotomy of DRG neurons led to increasing $Na_V1.3$ expression in injured neurons and frequency of neuron firing (10).

This study also showed that exercise training elevated miR-96 expression levels, which was accompanied by decreased $Na_V1.3$ expression in sciatic nerve in healthy and diabetic rats. In agreement with this study, Balducci *et al.* suggested that aerobic exercise training could alleviate peripheral diabetic neuropathy and even inhibit initiation of the disease (2). It has been known that beneficial effects of exercise were partially modulated by targeting miRNAs (11, 24, 33). Previous studies have also indicated that $Na_V1.3$ is linked with ectopic firing of neurons and neuropathic pain (9, 18). In this case, Samad *et al*. showed that $Na_V1.3$ knockdown in DRG of rats diminished neuropathic pain (30).

The positive role of exercise in diabetes man-

agement has previously been documented (7, 13, 22), as also in the present study that reported that swimming exercise protected diabetic rats from neuropathic pain. Moreover, previous studies reported that miRNAs are one possible mechanism of effects of physical exertion on body systems (23) and we, for the first time, showed exercise-dependent miR-96 elevation and $Na_V1.3$ reduction as a possible mechanism of exercise having positive influences on diabetic thermal hyperalgesia. Moreover, this study showed the beneficial effect of exercise on the miR-96/ $Na_V1.3$ link in healthy rats. It seems likely that miR-96-dependent effects of exercise is responsible for the reduction of sensitivity to thermal pain under healthy and diabetic conditions.

Taken together, this study reports the negative effects of diabetes on thermal hyperalgesia and down-regulated miR-96 expression as well as significant elevation of $Na_V1.3$ mRNA levels, which were both reversed by swimming training. It is, therefore, consided that miR-96/$Na_V1.3$ mRNA is an possible mechanism for protective effect of swimming exercise traning on diabetic thermal hyperalgesia and in healthy rats.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

References

1. Ahn, S. and Song, R. Effects of Tai Chi Exercise on glucose control, neuropathy scores, balance, and quality of life in patients with type 2 diabetes and neuropathy. *J. Altern. Complem. Med.* 18: 1172-1178, 2012.
2. Balducci, S., Iacobellis, G., Parisi, L., Di Biase, N., Calandriello, E., Leonetti, F. and Fallucca, F. Exercise training can modify the natural history of diabetic peripheral neuropathy. *J. Diabetes Complicat*. 20: 216-223, 2006.
3. Balducci, S., Leonetti, F., Di Mario, U. and Fallucca, F. Is a long-term aerobic plus resistance training program feasible for and effective on metabolic profiles in type 2 diabetic patients? *Diabetes Care* 27: 841-842, 2004.
4. Barrett, A.M., Lucero, M.A., Le, T., Robinson, R.L., Dworkin, R.H. and Chappell, A.S. Epidemiology, public health burden, and treatment of diabetic peripheral neuropathic pain: a review. *Pain Med.* 8: S50-S62, 2007.
5. Bartel, D.P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
6. Broderick, J.A. and Zamore, P.D. MicroRNA therapeutics. *Gene Ther*. 18: 1104-1110, 2011.
7. Castaneda, C., Layne, J.E., Munoz-Orians, L., Gordon, P.L., Walsmith, J., Foldvari, M., Roubenoff, R., Tucker, K.L. and Nelson, M.E. A randomized controlled trial of resistance exercise training to improve glycemic control in older adults with type 2 diabetes. *Diabetes Care* 25: 2335-2341, 2002.
8. Chen, H.P., Zhou, W., Kang, L.M., Yan, H., Zhang, L., Xu, B.H. and Cai, W.H. Intrathecal miR-96 inhibits $Na_V1.3$ expression and alleviates neuropathic pain in rat following chronic construction injury. *Neurochem. Res*. 39: 76-83, 2014.
9. Cheng, K.I., Lai, C.S., Wang, F.Y., Wang, H.C., Chang, L.L., Ho, S.T., Tsai, H.P. and Kwan, A.L. Intrathecal lidocaine pretreatment attenuates immediate neuropathic pain by modulating $Na_V1.3$ expression and decreasing spinal microglial activation. *BMC Neurol*. 11: 71, 2011.
10. Cummins, T.R., Aglieco, F., Renganathan, M., Herzog, R.I., Dib-Hajj, S.D. and Waxman, S.G. $Na_V1.3$ sodium channels: rapid repriming and slow closed-state inactivation display quantitative differences after expression in a mammalian cell line and in spinal sensory neurons. *J. Neurosci*. 21: 5952-5961, 2001.
11. Davidsen, P.K., Gallagher, I.J., Hartman, J.W., Tarnopolsky, M.A., Dela, F., Helge, J.W., Timmons, J.A. and Phillips, S.M. High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. *J. Appl. Physiol.* 110: 309-317, 2011.
12. Dobson, J., McMillan, J. and Li, L. Benefits of exercise intervention in reducing neuropathic pain. *Front. Cell. Neurosci*. 8: 102, 2014.
13. Dunstan, D.W., Daly, R.M., Owen, N., Jolley, D., De Courten, M., Shaw, J. and Zimmet, P. High-intensity resistance training improves glycemic control in older patients with type 2 diabetes. *Diabetes Care* 25: 1729-1736, 2002.
14. Goldhaber-Fiebert, J.D., Goldhaber-Fiebert, S.N., Tristán, M.L. and Nathan, D.M. Randomized controlled community-based nutrition and exercise intervention improves glycemia and cardiovascular risk factors in type 2 diabetic patients in rural Costa Rica. *Diabetes Care* 26: 24-29, 2003.
15. Guariguata, L., Whiting, D.R., Hambleton, I., Beagley, J., Linnenkamp, U. and Shaw, J.E. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res. Clin. Pract*. 103: 137-149, 2014.
16. Habib, A.A. and Brannagan, T.H. Therapeutic strategies for diabetic neuropathy. *Curr. Neurol. Neurosci. Rep.* 10: 92-100, 2010.
17. Habibi, P., Alihemmati, A., Nasirzadeh, M., Yousefi, H., Habibi, M. and Ahmadiasl, N. Involvement of microRNA-133 and -29 in cardiac disturbances in diabetic ovariectomized rats. *Iran J. Basic Med. Sci*. 19: 1177-1185, 2016.
18. Hains, B.C., Saab, C.Y., Klein, J.P., Craner, M.J. and Waxman, S.G. Altered sodium channel expression in second-order spinal sensory neurons contributes to pain after peripheral nerve injury. *J. Neurosci*. 24: 4832-4839, 2004.
19. Hong, S., Morrow, T.J., Paulson, P.E., Isom, L.L. and Wiley, J.W. Early painful diabetic neuropathy is associated with differential changes in tetrodotoxin-sensitive and -resistant sodium channels in dorsal root ganglion neurons in the rat. *J. Biol. Chem*. 279: 29341-29350, 2004.
20. Kluding, P.M., Pasnoor, M., Singh, R., Jernigan, S., Farmer, K., Rucker, J., Sharma, N. and Wright, D.E. The effect of exercise on neuropathic symptoms, nerve function, and cutaneous innervation in people with diabetic peripheral neuropathy. *J. Diabetes Complicat.* 26: 424-429, 2012.
21. Li, L. and Zhao, G. Downregulation of microRNA-218 relieves neuropathic pain by regulating suppressor of cytokine signaling 3. *Int. J. Mol. Med*. 37: 851-858, 2016.

22. Maiorana, A., O'Driscoll, G., Goodman, C., Taylor, R. and Green, D. Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes. *Diabetes Res. Clin. Pract*. 56: 115-123, 2002.
23. Makarova, J.A., Maltseva, D.V., Galatenko, V.V., Abbasi, A., Maximenko, D.G., Grigoriev, A.I., Tonevitsky, A.G. and Northoff, H. Exercise immunology meets MiRNAs. *Exerc. Immunol. Rev*. 20: 135-164, 2014.
24. Melo, S.F.S., Barauna, V.G., Neves, V.J., Fernandes, T., Lara, L.daS., Mazzotti, D.R. and Oliveira, E.M. Exercise training restores the cardiac microRNA-1 and −214 levels regulating Ca^{2+} handling after myocardial infarction. *BMC Cardiovasc. Disor*. 15: 166, 2015.
25. Mohajjel Nayebi, A., Sharifi, H., Ramadzani, M. and Rezazadeh, H. Effect of acute and chronic administration of carbamazepine on Cisplatin-induced hyperalgesia in rats. Jundishapur. *J. Nat. Pharm. Prod*. 7: 27-30, 2012.
26. Nath, K., Victor, R. and Naskar, S. Comparative study of various socio-demographic variables in patients having type 2 diabetes mellitus with or without depressive disorder: a brief report. *Open J. Psychiatry Allied. Sci*. 7: 142-148, 2016.
27. Ozkaya, Y.G., Agar, A., Hacioglu, G. and Yargicoglu, P. Exercise improves visual deficits tested by visual evoked potentials in streptozotocin-induced diabetic rats. *Tohoku J. Exp. Med*. 213: 313-321, 2007.
28. Peltier, A., Goutman, S.A. and Callaghan, B.C. Painful diabetic neuropathy. *BMJ* 348: g1799, 2014.
29. Rossi, D.M., Valenti, V.E. and Navega, M.T. Exercise training attenuates acute hyperalgesia in streptozotocin-induced diabetic female rats. *Clinics* 66: 1615-1619, 2011.
30. Samad, O.A., Tan, A.M., Cheng, X., Foster, E., Dib-Hajj, S.D. and Waxman, S.G. Virus-mediated shRNA knockdown of $Na_V1.3$ in rat dorsal root ganglion attenuates nerve injury-induced neuropathic pain. *Mol. Ther*. 21: 49-56, 2013.
31. Schreiber, A.K., Nones, C.F.M., Reis, R.C., Chichorro, J.G. and Cunha, J.M. Diabetic neuropathic pain: physiopathology and treatment. *World J. Diabetes* 6: 432-444, 2015.
32. Snedecor, S.J., Sudharshan, L., Cappelleri, J.C., Sadosky, A., Mehta, S. and Botteman, M. Systematic review and meta-analysis of pharmacological therapies for painful diabetic peripheral neuropathy. *Pain Pract*. 14: 167-184, 2014.
33. Soci, U.P., Fernandes, T., Hashimoto, N.Y., Mota, G.F., Amadeu, M.A., Rosa, K.T., Irigoyen, M.C., Phillips, M.I. and Oliveira, E.M. MicroRNAs 29 are involved in the improvement of ventricular compliance promoted by aerobic exercise training in rats. *Physiol. Genomics* 43: 665-673, 2011.
34. Winter, J., Jung, S., Keller, S., Gregory, R.I. and Diederichs, S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell. Biol*. 11: 228-234, 2009.
35. Yang, L., Li, Q., Liu, X. and Liu, S. Roles of voltage-gated tetrodotoxin-sensitive sodium channels $Na_V1.3$ and $Na_V1.7$ in diabetes and painful diabetic neuropathy. *Int. J. Mol. Sci*. 17: 1479, 2016.
36. Yousefzadeh, N., Jeddi, S. and Alipour, M.R. Effect of fetal hypothyroidism on cardiac myosin heavy chain expression in male rats. *Arq. Bras. Cardiol*. 107: 147-153, 2016.
37. Zhang, J., Zhang, H. and Zi, T. Overexpression of microRNA-141 relieves chronic constriction injury-induced neuropathic pain *via* targeting high-mobility group box 1. *Int. J. Mol. Med*. 36: 1433-1439, 2015.
38. Zhang, M., Lv, X.Y., Li, J., Xu, Z.G. and Chen, L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Exp. Diabetes Res*. 2008: 704045, 2008.