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Original Research

Modulation of oxidative and glycolytic skeletal muscle fibers Na^+/H^+ exchanger1 (NHE1) and Na^+/HCO_3^- co-transporter1 (NBC1) genes and proteins expression in type 2 diabetic rat (Streptozotocin + high fat diet) following long term endurance training

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Abstract: Diabetes is known to alter both oxidative and glycolytic pathways in a fiber type-dependent manner. The aim of present study was to investigate the effects of endurance training on muscle NHE1 and NBC1 genes and proteins expression in type 2 diabetic rats. Male wistar rats (n=30), 4 weeks old and 95.7±10.8g, were randomly selected and divided into control, diabetic without training and diabetic with training groups. Diabetes was induced by injection of low dose of streptotozin and feeding with high-fat diet. The Endurance training was performed for 7 weeks that started with relatively low speed and duration of 20 m min^{-1} for 20 min in the first week and gradually reached to 30 m min^{-1} for 35min in the last week. NHE1 and NBC1 genes and proteins expression were determined by Real time-PCR and western blotting techniques, respectively, in Soleus as an oxidative and EDL (Extensor digitorum longus) as a glycolytic muscle preparation. NHE1 mRNA and protein expression reduced significantly in EDL and Soleus in the diabetic without training group compared with the control group. However, reduction in the expression of NBC1 gene and protein in the diabetic without training group compared to controls did not significant. Endurance training increased NHE1 and NBC1 genes and proteins expression in both EDL and Soleus in the diabetic training group compared to control groups. In conclusion, endurance training may improve the capacity of pH_i regulation in muscles by lactate-independent pathway.

Key words: Gene expression; Protein expression; NHE1, NBC1; Endurance training; Type 2 diabetes.

Introduction

Over recent years, due to inactivity, the prevalence of diabetes, specially type 2 diabetes mellitus (T2DM), has significantly increased (1, 2). T2DM leads to hyperglycemia, increased lipolysis and gluconeogenesis and, eventually, in severe conditions, diabetic ketoacidosis may occur. In diabetic ketoacidosis the cellular pH reduces and H^+ accumulates(3). Decreased intracellular pH may also change the protein conformation, may alter channel properties(4), and may reduce the activity of key enzymes of glycolysis with subsequent reduced rate of ATP re synthesis (5, 6). Consequently, it may be crucial for the diabetic muscle cells to delay pH decline in the cytosol. The cellular mechanisms of proton buffering represent the first line of defense against acidosis. Elevated muscle buffering would enable the muscle to produce more protons before reaching pH_i limits(7). On the other hand, releasing lactate and H^+ ions to the outside and HCO_3^- to the inside of the muscle cell is another protective mechanism against intracellular pH decrease(8).

Na/ H^+ exchangers and Na/HCO_3 co-transporters are the classical pH-regulating transport systems presented as lactate-independent H/transport system in many cell types (9-17). Also, NHEs, NBCs with transport of Na and bicarbonate (1:2) help to keep pH_i constant in cell (11, 18). Although, at rest Na^+/H^+ exchange is considered as the most important system in regulating pH_i, bicarbonate transport is probably involved.

There are limited data regarding the training-induced changes in NHE1, NBC1 mRNAs and proteins expression in skeletal muscle in patients with T2DM. However, in the myocardium of streptozotocin- induced diabetic rats there was an altered pH regulation and a reduced Na^+/H^+ exchanger capacity (19, 20). Furthermore, the NHE1 expression in rats skeletal muscle increased after 6 weeks high-intensity treadmill training (21, 22). It has been demonstrated that sprint training increased the NHE1 mRNA in oxidative fibers (Soleus) but not in glycolytic fibers (EDL) but endurance training had no effect on NHE1 mRNA in both Soleus and EDL fibers (23). In contrast, NBC1 expression has been shown to be elevated after high-intensity training in oxidative skeletal muscle (14), but there is no report on NBC1 mRNA expression after resistance and endurance training. Thus, it seems that the type of training intensity and specificity of fiber-type are key factors for fluctuations in mRNA and protein expression of NHE1 and NBC1. Therefore, the aims of the present study were (1) to investigate the effect of endurance training on NHE1 and NBC1 genes and proteins expression in muscle samples from type 2 diabetic rats with and without training and matched healthy control, (2) to compare NHE1

and NBC1 genes and proteins expression in oxidative and glycolytic muscle fibers in three groups.

Materials and Methods

Ethical approval

All the experimental procedures performed in this study were approved by the Ethical Committee of the Tarbiat Modarres University.

Animals

30 male Wistar rats, 4 weeks old, with a mean body weight of 93/7±8/9g (Pastor Institute of Iran), were caged in temperature condition of 22±4 °C under 12:12 dark-lighting cycle. After 2 weeks, when all rats reached the average weight of 183/47±11/4g, they were matched and randomly divided into control (n=10), diabetic without training (diabetic control) (n=10) and diabetic with training (n=10) groups(24). To develop a rat model of type 2 diabetic mellitus, diabetic groups were injected with low-dose (35mg/kg) STZ after high-fat diet (HFD) (58% calories as a fat) (Table 1) while the control group were fed normal pellet diet (NFD) (12% calories as a fat) for two weeks. 48 hours after STZ injection, blood samples were collected and glucose concentration was measured by enzymatic glucose-oxidase technique. The rats with high blood-glucose concentration (>300 dl/mg) were eligible for the study (23, 25, 26).

HOMA-IR test

24 h after the last training session, blood samples (1ml) were collected from the eyes of the animals for measuring plasma insulin, glucose and HOMA-IR index. Plasma insulin was measured by ELISA technique with sensitivity of 1ng ml^{-1}, according to the manufacturer's instructions. HOMA-IR values were also calculated using the formula of HOMA-IR=Insulin [μUml^{-1}]×Glucose [$mmoll^{-1}$]/22.5(1, 27).

Insulin resistance was subject to two conditions:(1) HOMA-IR values above 2.5 and (2) higher fasting insulin levels >160 pmol (1, 27). Only rats in diabetic control and diabetic training groups with the above-mentioned conditions were included in the final analysis.

Training

Rats in the diabetic training group were trained once a day, 6 days a week, for a period of 7 weeks on a rodent motor driven treadmill equipped with electrical stimulus system. Each training session consisted of one continues bout without rest with low speed and duration and (20 $mmin^{-1}$ and 20 min, respectively) in the first week and were gradually increased to higher speed and duration (30 $mmin^{-1}$ and 35 min) in the last week (Table 2). The latter intensity demands an oxygen uptake of about 50-60 $mlkg^{-1}min^{-1}$ which corresponds to the sub maximal oxygen uptake (28, 29). Serum lactate level (measured in pilot study) was approximately 4 $mmoll^{-1}$ during the speed of 30 $mmin^{-1}$. All the training variables were kept constant in the last two weeks of the training sessions.

Table 1. Composition of high-fat diet (HFD) that used in this study.

Ingredients	Diet(g/kg)
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
DL-methionine	03
Yeast powder	01
Sodium chloride	01

Sample extraction

Forty eight hours after the last training session, the rats were anesthetized by intraperitoneal injection of ketamine (90 $mgkg^{-1}$) and xylazine (10$mgkg^{-1}$) then were sacrificed. The muscle samples (Soleus and EDL) were immediately removed and frozen in liquid nitrogen for subsequent analyses (25, 30).

RNA Isolation and Reverse Transcription

Total RNAs from the muscles were isolated using Biozol reagent following the manufacturer's guidelines. The final pellets were resuspended in 50-µl diethyl pyrocarbonate-treated H_2O containing 0.1 mM EDTA. RNAs were quantified by measuring the absorbance at 260 nm, and the purity of the samples was assessed by the absorbance at 260- to-280-nm ratio, which was >1.9. The integrity of the RNAs was confirmed by visual inspection of the 18S and 28S RNA bands on an ethidium bromide-stained formaldehyde agarose gel. Reverse transcription (RT) of total RNAs (3 µg) were performed using the Superscript II RNase system (Invitrogen, Carlsbad, CA) (23, 31). Each RT sample was diluted in nuclease-free water to a total volume of 50µl.

Normalization of mRNA Expression

For normalizing mRNA expression, the complementary DNA (cDNA) content was assessed in each sample using OliGreen reagent, as previously described (23, 31).Thus, the mRNA is expressed relative to the sample cDNA content.

Real Time PCR

The mRNA content of the selected genes was determined by real time PCR. Forward and reverse primers and probes (18s) were designed from rat-specific sequence databases using computer software (Table 3).

For each gene, a Blast search revealed that sequence homology was obtained only for the target gene. Optimization and PCR were performed as previously described (23, 31). Samples were analyzed in triplicate, and the mean inter assay coefficient of variation was <2% for all the target genes (Table 4). The threshold cycle, reflecting the initial target mRNA content in the sample,

Table 2. Endurance training protocol for a period of 7 weeks.

	Acquaintance 5 days	First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week
Speed ($mmin^{-1}$)	15	20	20	25	25	30	30	30
Duration (min)	20	20	25	25	30	30	35	35

Table 3. Primers used in Real Time PCR method.

Gene bank	Reverse primer	Forward primer	gene
Slc9a1	GCTGGCAAACTCCTCAAAG	CACATCAATGAGCTGCTGC	NHE1
Slc4a4	CATGGTAGGACTTGGCTTTC	ACTCCCTTCATTGCCTTTG	NBC1
18s	GTTGGTTTTCGGAACTGAGGC	GTC GGC ATC GTT TAT GGT CG	18S

Table 4. PCR conditions for amplification of NHE1, NBC1 and 18S mRNA.

Product	Syber mix (µl)	Primers (µl)	Taq-polymerase (µl)	cDNA (µl)	DDW (µl)
NHE1	12.5	0.50	0.15	2	9
NBC1	12.5	0.50	0.15	2	9
18S	12.5	0.50	0.15	2	9

was converted to a relative amount using a standard curve obtained by running a serial dilution of a pooled RT sample, together with the samples. Then, target gene expressions were evaluated by $2^{-\Delta\Delta ct}$ (23, 31).

Western blotting

30 mg of each muscle sample was powdered using a ponder, homogenized in 2ml of sucrose buffer (250 mM sucrose, 30 mM HEPES, 2mM EGTA, 40 mM NaCl, 2 mM phenylmethylsulphonfluoride (PMSF), pH 7.4) and centrifuged at 1000 *g* for 5 min. The supernatant was again centrifuged at 230000 *g* for 180 min at 4°C in a high-speed centrifuge equipped with a swing-out bucket rotor. The resulting pellet (corresponding to the membrane fraction) was resuspended in Tris-SDS (10 mM Tris, 4% SDS, 1 mM EDTA, 2mM PMSF, pH 7.4). Protein expression determined with a bovine serum albumin (BSA) standard (DC protein assay, Bio-Rad) and the samples were diluted to a protein concentration of 1 mg mL^{-1}. The protein samples (30 µg) were run on to SDS–PAG (8–18% gradient gel). The separated proteins were electro-blotted onto a Millipore Immobilon-P polyvinylidene difluorid membrane. The membrane was blocked in TBS buffer (10 mM tris-base, 0.9% NaCl, pH 7.4) containing 1% (wv^{-1}) BSA, 0.5% low fat dry milk and 0.1% Tween-20, and incubated at 4°C overnight in the primary antibody diluted 1:1000 in TBS buffer containing 1% BSA and 0.5% low fat dry milk. After washing (3×15 min) with TBST, the membrane was incubated in the secondary antibody (HRP-anti-Rabbit immunoglobulins, AP7181, FARATEB) for 1 hour at dilution of 1:10000. The membrane was washed (3×15min) with TBS and then incubated with ECL reagents (Amersham) and visualized on a film. The quantification of NHE1 and NBC1 proteins was performed by scanning the film and analyzing band intensities using Image J Software (14, 23, 25).

Antibodies

Both NHE1 and NBC1 antibodies with given codes of ab67313 and ab30322, respectively were purchased from ABCAM Company in Cambridge, England. Secondary antibody was purchased from FARATEB RAZI Company in Tehran, Iran. A sample of NBC1 and NHE1 was used as control together with rat-erythrocyte ghosts.

Calculations and statistics

Data of mRNA analysis are reported as fold changes and standard errors (SE) of the mean. Values of protein expression densitometry are expressed as percentage of sample values compared to control value (mean ± SE). The differences in mRNA variables between groups were determined using the One-way ANOVA. Two muscle samples (Soleus and EDL) from each rat were run on the same gel allowing a direct comparison of NHE1and NBC1 density between the muscles. In order to be able to compare the rats, some series of samples from different rats were compared after normalization assuming identical mean NHE1 and NBC1 density of the two muscles. Linear regression and calculation of the statistically significant levels were obtained using Excel and SPSS 16. One-way ANOVA was used to evaluate the differences between the mean values of protein density. The significant level ($P< 0.05$) was considered in data-analysis section of the study.

Results

Our results showed (Table5) that after four weeks of the onset of high-fat diet consumption by diabe-

Table 5. Effects of 7 weeks endurance training on insulin resistance indices and skeletal muscle biomarkers (mean, SD;n10).

Variable	Control	Diabetic control	Diabetic trained
Weight			
Pre	190±10	190±12	185±9
Post	297±11	330±20*	290±13*
Glucose			
Pre	109.4±12.58	354.25±29.15*	385±33.7*
Post	106.4±9.26	394.44±48.74*	301.7±47.95*
Insulin	6.28±1.15	10.55±1.12*	8.95±0.78*
HOMA-IR	1.63±0.24	10.48±1.44*	6.68±1.31*
Soleus NHE1 mRNA	1	0.81±0.06*	0.87±0.07
EDL NHE1 mRNA	1	0.75±0.07*	0.93±0.09
Soleus NBC1 mRNA	1	0.71±0.07*	0.75±0.07*
EDL NBC1 mRNA	1	0.65±0.07*	0.81±0.68
Soleus NHE1 expression	3.75±0.30	2.81±0.25*	3.42±0.41
EDL NHE1 expression	4.71±0.21	3.69±0.51*	4.9±0.68
Soleus NBC1 expression	3.84 ±0.33	2.93±0.35	3.51 ±0.62
EDL NBC1 expression	2.15±0.69	1.88±0.12	1.99±0.51

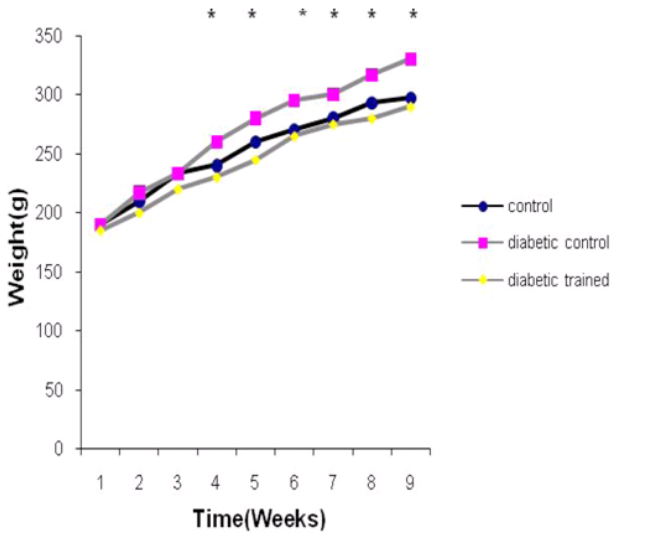

Figure 1. Changes in body weight after HFD consumption. Control (n=10), diabetic control (n=10), diabetic trained (n=10). *Significantly difference compared with the diabetic control (P<0.05).

tic groups, the amount of weight gained in the diabetic control group and the other groups was different (p<0.05), and this difference remained significant until the end of the study (Figure 1).

The results also demonstrated a significant increase in blood glucose levels (Pre glucose), insulin and HOMA-IR index in the diabetic groups than those in the control group (Figure 2 and 3), indicating that diabetic model has been applied correctly.

All muscles NHE1 and NBC1 mRNA and protein expressions were also standard in the diabetic control and the training groups compared with those in the control group. The results of the current study indicated that in the presence of T2DM the NHE1 mRNA expression significantly decreased in Soleus and EDL muscles by 19 and 25%, respectively, in the diabetic control group compared with healthy rats (Figure 4).

After seven weeks of endurance training, NHE1 mRNA expression increased (Figure 4) in Soleus and EDL by 6 and 18%, respectively, in the diabetic trained group compared with those in the diabetic control group (P <0.05). Further, we detected that in the presence of T2DM the NBC1 mRNA expression significantly decreased (Figure 5) in Soleus and EDL by 30 and 35%,

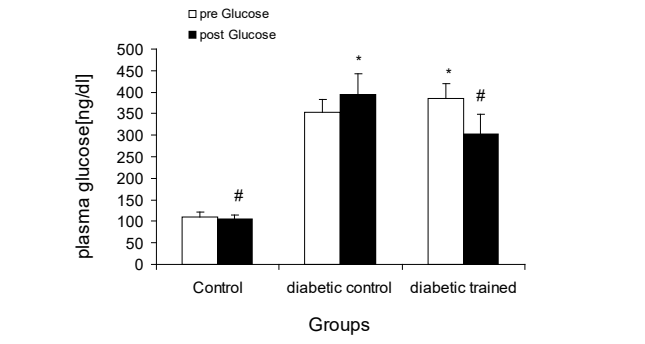

Figure 2. Changes in plasma glucose before and after endurance training. Control (n=10), diabetic control (n=10), diabetic trained (n=10).* Significantly difference compared with control (P<0.05). # Significantly difference compared with diabetic control (P<0.05).

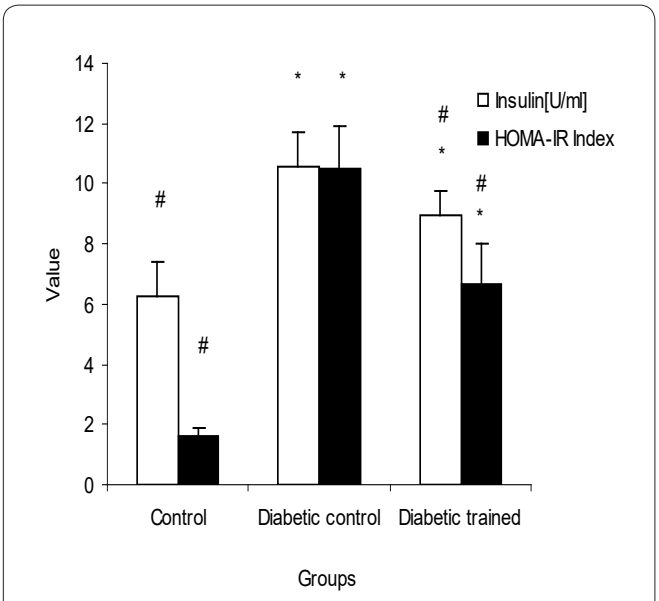

Figure 3. Changes in plasma insulin and HOMA-IR index before and after endurance training. Control (n=10), diabetic control (n=10), diabetic trained (n=10). * Significantly difference compared with the control (P<0.01). # Significantly difference compared with the diabetic control (P<0.01).

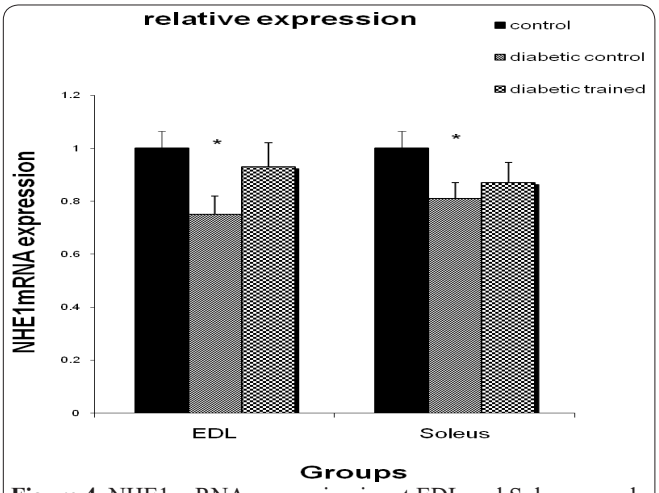

Figure 4. NHE1 mRNA expression in rat EDL and Soleus muscle fibers. Control (n=10), diabetic control (n=10), diabetic trained (n=10). *Significantly difference compared with control (P<0.05).

respectively, in the diabetic control group compared with those in the control group (P <0.05).

Also, we found that the endurance training increased NBC1 mRNA expression (Figure 5) in Soleus and EDL by 5 and 16%, respectively, in the diabetic trained group compared with those in the diabetic control group (P <0.05). Also, analyzing protein expression using the antibodies identified a protein with molecular mass of 91 kDa (NHE1) and a protein with molecular mass of 120 kDa (NBC1) in muscle homogenates. Also, the presence of T2DM significantly decreased the expression of NHE1 in Soleus and EDL by 24 and 22%, respectively, in the diabetic control group compared with those in the control group (P <0.05). Endurance training increased NHE1 expression (Figure 6) in the diabetic trained group by 15 and 26% in Soleus and EDL muscles, respectively compared with those in the diabetic control group (P <0.05).

We have also found that in the presence of decreased NBC1 expression (Figure 7) in Soleus and EDL by 23

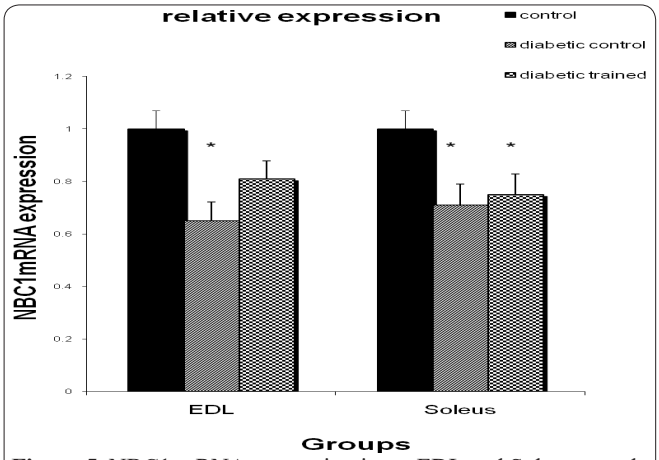

Figure 5. NBC1 mRNA expression in rat EDL and Soleus muscle fibers.Control (n=10), diabetic control (n=10), diabetic trained (n=10). *Significantly difference compared with control (P<0.05).

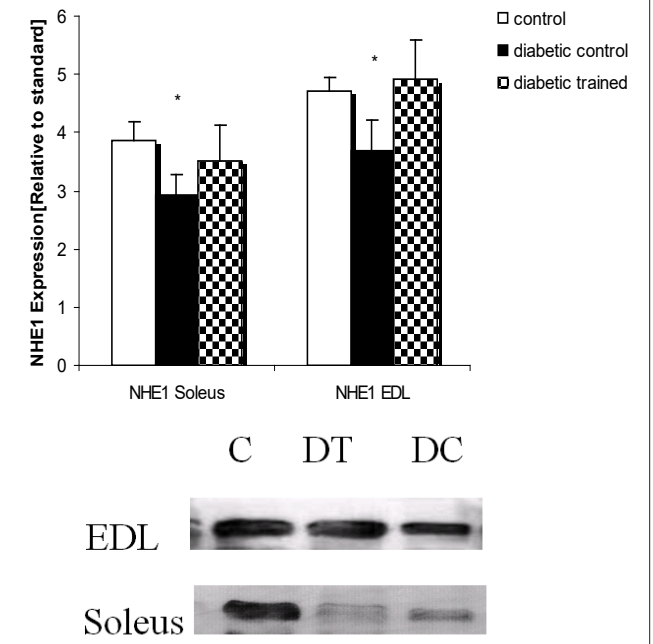

Figure 6. NHE1 protein expression in rat EDL and Soleus muscle fibers. Control (C, n=10), diabetic control (DC, n=10), diabetic trained (DT, n=10) *Significantly difference compared with control (p<0.05).

and 13% in the diabetic control group compared with the control group, respectively (P >0.05). Also, the expression of NBC1 increased (Figure 7) in Soleus and EDL by 18% and 6%, respectively in the diabetic trained group compared with the diabetic control group after the endurance training (P <0.05).

Discussion

The major findings of the present study were that NHE1 and NBC1 mRNAs and proteins expression decreased in the diabetic control group compared with the other diabetic groups and the endurance training increased NHE1 and NBC1 mRNAs and proteins expression in the diabetic trained group compared with the other diabetic groups, leading to normalizing mRNAs and the protein contents in the latter group. The present study is the first one to evaluate the long-term effects of the endurance training (30 m min^{-1} and 35 min) on the skeletal muscle NHE1, NBC1 mRNAs and proteins

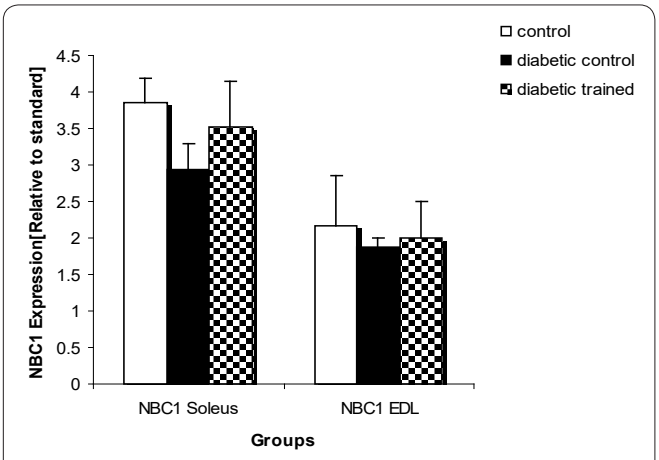

Figure 7. NBC1 protein expression in rat EDL and Soleus muscle fibers. Control (C, n=10), diabetic control (DC, n=10), diabetic trained (DT, n=10).

expression in type 2 diabetic rats.

These findings showed that, NHE1 and NBC1 mRNAs and proteins expression are altered by the endurance training in a muscle group-specific way. Changes in body weight (Figure 1), blood glucose (Figure 2), insulin levels (Figure 3) and HOMA-IR index (Figure 3) after the consumption of high-fat diet (HFD) and streptozotocin (STZ) injection in all the diabetic groups, indicated that the model of type 2 diabetic rats has been applied correctly. The results of the study revealed that NHE1 mRNA expression significantly decreased in Soleus and EDL muscles in the diabetic control group in comparison with the healthy rats, respectively. Endurance training increased NHE1 mRNA expression in Soleus and EDL in the diabetic trained group in comparison with the control group. Also, the NBC1 mRNA expression significantly decreased in Soleus and EDL in the diabetic control compared with healthy rats, and endurance training modulated NBC1 mRNA expression in Soleus and EDL in the diabetic trained group. These findings are in accordance with the reports that demonstrated coronary artery ligation causing the elevation of NHE1 and NBC1 mRNA levels in type 2 diabetic rats (31-33). However, in a study, Peirce et al. (1990) showed that NHE1 gene expression did not increase in heart rats after STZ administration. We indicated that there is greater mRNA expression in NHE1 compared with NBC1 in the diabetic trained group that might demonstrate the importance of NHE1 in pH regulation.

Further, our findings are in accordance with report of Le Prigent et al. (1996) that showed in normal adult myocytes, the contribution of the total efflux of H^+ equivalents of the Na^+/H^+ exchanger and of the Na^+/HCO_3^- co-transporter to be 69 and 31%, respectively at pH 6.90 (34). Also, we found significantly decreased NHE1 protein expression in both Soleus (32%) and EDL (34%) of the diabetic control group compared with the other diabetic groups; and detected that the endurance training increased NHE1 protein expression in both Soleus and EDL in the diabetic trained group compared with the other diabetic groups group. In accordance with these findings, Le-prigent et al. (1997) also depicted that NHE1 mRNA and protein expression decreased after STZ administration in heart rats. However, Darmellah et al. (2007) showed that NHE1 activity increased in type 2 diabetic rats. Moreover, the data of the present

study showed that endurance training caused a large increase in NHE1 mRNA expression with a proportional increase in protein expression in the diabetic trained group. We have also shown that type 2 diabetes decreased NBC1 expression in Soleus and EDL by 13 and 24% in the diabetic control group, respectively compared with the other diabetic groups group. Furthermore, endurance training alleviated the reductions in NBC1 content in both Soleus and EDL in the diabetic trained group. It is often assumed that increased mRNA expression causes increased protein de novo synthesis. However, numerous factors such as protein degradation rate, posttranscriptional modifications and mRNA stability can also affect the relation between mRNA and protein expression (23). In the present study, we confirm that NBC1 mRNA is expressed in both oxidative and glycolytic skeletal muscle fibers while greater training induced increase in NBC1 expression in oxidative muscle fiber. In contrast, the increase in NBC1 mRNA expression in both Soleus and EDL was not proportional to an increase in protein expression in the diabetic trained group. The reasons for this discrepancy are not known, but some posttranscriptional modifications may be involved.

The effect of endurance training in alteration of NBC1 and NHE1 mRNAs and proteins content in EDL and Soleus can suggest the posttranslational modifications and fiber –type specific response of these proteins in the diabetic trained group. The signals initiating the training-induced changes of these transporters expression are not known, but mode and the intensity of training for production of proton and lactate may suggest acidification associated with T2DM and training could be a signal involved in the increase of NHE1 and NBC1 mRNAs and proteins expression in type 2 diabetic rats (14, 23, 35).

However, other factors related to muscle activity could be involved as well, and the changes in NHE1 and NBC1 expression may be part of a general response to the repeated exercise sessions. In accordance with the present findings and interpretations, Juel (1998) reported that high-intensity exercise training by treadmill, 3×3 min, 40 $mmin^{-1}$ for 6 weeks significantly increased NHE1 activity but the endurance training on treadmill, 20 $m.min^{-1}$, 40 min for 6 weeks had no effect on NHE1 activity in rat skeletal muscle. In addition, Rasmosen et al. (2011) reported that one session sprint training increased NHE1 mRNA expression in rat. They also reported that after 3 weeks of endurance training NHE1 mRNA expression increased but NHE1 protein expression did not change. Lack of changes in NHE1 following resistance and the endurance training suggests that this alteration is a fiber-specific response. These data suggest that high-intensity exercise, with its concomitant large changes in pH, is needed to increase NHE1 density, while resistance and endurance training which causes only moderate pH changes, are insufficient to change NHE1 density (14).

Further, our findings suggest that adaptations are specific to the way of training. However, the endurance training in the present study (30 $mmin^{-1}$) demanded an oxygen uptake of about 50-60 $mlkg^{-1}min^{-1}$, which corresponds to sub maximal oxygen uptake (28). Thus, the speeds applied in the present study must be expected to stimulate both the aerobic and anaerobic systems. Therefore, lactate accumulation and decreased pH are expected. The result showed that severe metabolic perturbation (e.g. disturbance of proton homeostasis as a result of T2DM and training) is required for NHE1 and NBC1 proteins expression to be altered. This result would imply that NHE1expression was influenced by cellular metabolites changes. In contrast, Dela *et al*. (2004) used 4 sets of intermittent one-leg press resistance training with 70-80 1RM, 8-12 rep and 90s rest between each set. The result showed that content of Na/K pump components tended to be lower in patient with type2 diabetes compared to control subjects and training prevent the decrease in pump content components associated with diabetes in trained diabetic leg. The study of the three subunits (α_1,α_2 and β) of the Na+,K+ pump revealed a very similar pattern, a reduction in diabetes and an increase with training. It could therefore be argued that the effect was due to a general and unspecific reduction in membrane function(36).

We also showed that NHE1 is expressed in all fiber types but mostly in glycolytic fibers in the diabetic trained group. The highest NHE1 density in glycolytic fibers indicate better capacity for pH regulation, which is consistent with the larger capacity for production of lactic acid in these fibers (data are not shown). Similar to NHE1, we report that NBC1 responds to a fiber –type specificity to the endurance training (i.e. NBC1 is expressed mostly in oxidative fiber but not significantly). Thus, we have shown that like other membrane transport proteins involved in muscle pH regulation (i.e. MCTs and NHEs), NBC1 is sensitive to chronic contractile activity in rat skeletal muscle fibers. The results of the present study are in accordance with the finding of Thomas & David (2007) that reported an increased NBC1expression in oxidative fiber after high–intensity training. In contrast, Juel et al. (2003) found the increased expression of NBC1 in type II fibers after hypoxic condition. Data on NBC1 responses to the exercise training are contradictory; however, Kristensen et al. (2004) showed that there is no correlation between NBC1 and fiber- type and this transporter express in all fiber types.

In conclusion, this study depicted that NHE1, NBC1 mRNAs and proteins expression decreased in rat skeletal muscles in the diabetic control group and the endurance training increased both NHE1 and NBC1 mRNAs and proteins expression in the diabetic trained group leading to normalizing the mRNAs content in the diabetic trained group. The results also showed that NHE1 and NBC1 gene and protein expressions undergo fiber specific adaptive changes in response to mode and the intensity of the training (endurance training). Consequently, we can speculate that proton transport may be facilitated by NHE1 and NBC1, thereby endurance training may improve the capacity for pH regulation in skeletal muscle of type 2 diabetic rats by independent lactate pathway and these increases depend on a transporter-type specificity which is mostly important in pH regulation.

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Disclaimer

Authors certify that there is no actual or potential conflict of interest in relation to this article. Note that part of our manuscript has previously published in a local journal in Persian language (Sport Physiology, No 22).

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