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The Effect of resistance exercise training on calcineurin signaling expression in skeletal muscle of diabetic rats

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ABSTRACT

The Effect of resistance exercise training on calcineurin signaling expression in skeletal muscle of diabetic rats was studied. Male Wistar rats were randomized to one of four groups: control (C), trained (T), STZ-induced diabetes (D), and (Streptozotocin) STZ-induced diabetes plus training (DT). Diabetes was induced by the i.p. injection of STZ (Sigma), 55 mg/kg of body weight in a 0.1 M citrate buffer (pH 4.5). The results showed that, Typical Type 1(diabetes mellitus) DM hyperglycemia occurred in D and DT groups with increased fasting glucose (P<0.001). Moreover, diabetic and resistance training rats showed a significant decrease in total weight (P<0.001). Significant different observed among groups in expression of(Regulator of calcineurin1) RCAN-1 mRNA (P<0.05). As RCAN-1(messenger ribonucleic acid) mRNA increased in T group compare to C group (P<0.05). There was a significant decrease of RCAN-1 mRNA in soleus muscle D group compared with C group (P<0.05). There was not difference in DT group compared with C and D groups (P>0.05).

Key words: Diabetes; Calcineurin Signaling; Resistance Exercise; Hyperglycemia

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INTRODUCTION

Diabetes mellitus is a chronic disease characterized by elevated plasma glucose concentration resulting from insulin insufficiency and/or insulin resistance [1]. Disease management includes lifestyle modifications, diet, exercise, and long term use of oral hypoglycemic agents or insulin therapy [2]. Obvious muscle atrophy is a characteristic of type 1 diabetes [3]. It shown that endurance exercise training could diminish the skeletal muscle wasting in diabetic rats with muscle atrophy [4]. Hypertrophy of skeletal muscle and its concomitant gains in power are of great interest to people from disease-induced atrophy. Resistance exercise training has shown to be an effective strategy to augment muscle mass, strength and function in diabetes [5]. It proposed increased glucose uptake observed after resistance training in due to increase in muscle mass. The calcineurin has been implicated as a molecular decoder of the sustained intracellular Ca2 signals evoked in muscle cells in response to motoneuron activation. In particular, the calcineurin activates nuclear trans-acting factors such as(Nuclear factor of activated T-cells) NFATc [6]. The calcineurin has been implicated in several adaptive responses inducing muscle fiber growth/regeneration and slow

fibre-type gene expression [7]. The calcineurin appears to act as a co regulator of muscle hypertrophy with IGF and may also contribute to myogenic proliferation and differentiation of satellite cells during skeletal muscle regeneration [8]. Use of the calcium/calmodulin inhibitors suppresses growth of overloaded muscle fibers, while the calcineurin over expression reduces disuse atrophy [7]. In transgenic mice provided evidence that skeletal muscle remodeling to a slow-twitch phenotype via constitutively active calcineurin enhanced insulin-stimulated glucose transport in diabetic rats [9]. Roberts and coworker's in chronic STZ induced rats showed protein calcineurin and signaling pathway for calcineurin were decreased [10]. A family of proteins conserved from yeast to humans, termed(endogenous regulators of calcineurin (RCNs)) RCNs, was recently identified as endogenous regulators of calcineurin [11,12]. In budding yeast, overexpression of either Rcn1 or two of its human homologs(Down syndrome critical region gene 1) DSCR1/MCIP1(Modulatory Calcineurin Interacting Protein 1) and(Calcineurin inhibitory protein) ZAKI4/MCIP2 (Modulatory Calcineurin Interacting Protein 2strongly inhibited calcineurin-dependent processes [12]. Similarly, overexpression of DSCR1/MCIP1 inhibited calcineurin-dependent activation of NFAT and other transcription factors [13]. DSCR1/MCIP1 overexpression in the developing heart also blocked calcineurin-induced cardiac hypertrophy in mouse [14]. RCNs directly interact with calcineurin in vivo, and purified recombinant DSCR1/MCIP1 can bind to the catalytic subunit of calcineurin in vitro and potently inhibit the protein phosphatase activity of the holoenzyme [12-15].

RCNs seem to stimulate calcineurin signaling when expressed at their physiological levels. Expression of RCN1 and DSCR1/MCIP1 genes are strongly up-regulated in response to calcineurin signaling [13]. The effect of resistance training on signaling calcineurin in diabetes after resistance training never been investigated. Cleared that calcineurin has been characterized as an anabolic factor in skeletal muscle. It was hypothesized that this factor would maintain with resistance training in diabetes.

MATEREALS AND METHODS

Animals

All experiments involving the animals were conducted according to the policy of Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and the protocol was approved by the Ethics Committee of the School of the Medicine Sciences, Tarbiat Modares University (TMU), and Tehran, Iran. The Male Wistar rats weighing 250–280 g, were used in this study and were housed in a light and temperature-controlled animal facility with free access to tap water and food pellets.

Animals were randomly assigned to the treatment groups: control (C), trained (T), STZ-induced diabetes (D), and STZ-induced diabetes plus training (DT). Diabetes was induced with the i.p. injection of STZ (Sigma), 55 mg/kg of body weight in a 0.1 M citrate buffer (pH 4.5). An equal volume of buffer was injected into the control rats. Blood glucose concentration was assessed after 4 days to ensure that fasting levels greater than 14 mmol/l (250 mg/dl) were reached. The diabetic rats were not treated with insulin during the study, and they showed symptoms of type 1 diabetes, such as polyuria and weight loss. Fasting blood was sampled from tail vein after overnight fast. Blood glucose levels were tested by glucometer (GT-1920, Japan)

Resistance Training

The rats in the T and DT groups were trained using a protocol previously described [16]. Briefly, animal climbed 26 rugs across the 1 m ladder. One repetition along the ladder required 26 total lifts by the animal (or 13 lifts per limb). The rats were familiarized with the exercise for three days, 48 h before STZ injection. Rats were positioned at the bottom of climbing apparatus and motivated to climb the ladder by touching the tail.

The exercised animals trained 5 weeks with a rest of 48 h between sessions. Animals from the T and DT groups were exercised with 5 sets of 4 repetitions each with a 60 s rest interval between the reps and 3 min between the sets at per session. T group started with 50% body mass (BM) at the first three sessions. At 4 to 6 sessions they were carrying 80% BM. At 7 to 9 sessions they were carrying 100% BM. At 10 to 12 sessions they were carrying 120% BM. At 13 and 14 sessions they had a load decrease that were carrying 120% BM with 3 sets of 5 repetitions. At 15 to 17 sessions they were carrying 150% BM. At the similar sessions of DT group carrying 30%, 50%, 80%, 100% and 120% BM. Also, they had similar load decrease that carrying 100% BM with 3 sets of 5 repetitions.

Tissue preparation
Twenty-four hours after the last training session, rats were anesthetized intraperitoneally with a mixture Ketamine™ (30–50 mg/kg bw, ip) and Xylazine (3–5 mg/kg bw, ip), the soleus muscle was quickly extracted, weighed, and stored in liquid N_2 for posterior analysis.

Real Time PCR
Total RNA was extracted from frozen soleus and FHL muscles samples using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA) following the manufacturer's recommendations. Concentrations of RNA were determined by measuring absorbance at 260 nm. The purity of the RNA was determined by calculating the absorbance ratio at 260 and 280 nm, and by ethidium bromide staining. Purification was accepted when the absorbance 260/280 nm ratio was above 1.8. Isolated RNA was stored at 80°C. RNA was reverse transcribed into complementary DNA (cDNA) using a PrimeScript RT reagent Kit (Perfect Real Time, Takara Code RR037A, Japan) using the following protocol: reverse transcription at 37 ° C for 15 minutes, inactivation of reverse transcriptase at 85°C for 5 s, and refrigeration at 4°C for 10 minutes. It was then stored at 20°C.

Primer sets for rat GAPDH (glyceraldehyde 3-phosphate dehydrogenase), RPL-26 (ribosomal protein L26) and RCAN-1 were produced by Qiagen Tect (GAPDH, QT00199633, RPL-26, QT01828771 and RCAN-1, QT00181293). Gene expression was measured by real-time PCR using the Rotor-Gene 6000 (Corbett Research, Mortlake, Australia). The thermal cycle protocol was as follows: 1 cycle at 95 C for 30 s, 40 cycles at 95 C for 5 s and 60°C for 30 s. PCR amplification was performed in duplicate in a total reaction volume of 20 µl. The reaction mixture consisted of 3 µl diluted template, 10 µl the SYBR Premix Ex Taq™Kit (Perfect Real Time, Takara Code RR041A, Japan), and 2 µl primers. Amplification specificity was controlled by a melting curve analysis and a gel electrophoresis of the PCR product.

Relative expression levels of RCAN-1 were normalized by subtracting the corresponding levels of mean of GAPDH and RPL-26 ΔCT, which were amplified as housekeeping genes. All data are represented relative to its expression as fold change from C group (17).

Blood glucose Measurement
Serum fasting glucose level were determined using a glucose oxidase kit glucose B-test, (Wako Chemicals, Japan) with samples run in duplicate.

Statistical analysis
All analyses were performed using SPSS V16.0 (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) and LSD post hoc test used for real time and protein data. Statistical significance was set at $p<0.05$. Data are presented as means ± SEM.

RESULTS AND DISCUSSION

Typical Type 1 DM hyperglycemia occurred in D and DT groups with increased fasting glucose ($P<0.001$; Table 1). Moreover, diabetic and resistance training rats showed a significant decrease in total weight ($P<0.001$; Table 1).

Significant different observed among groups in expression of RCAN-1 mRNA ($P<0.05$). Post-hoc analyses indicated RCAN-1 mRNA increased in T group compare with C group ($P<0.05$) (Figure 1). There was a significant decrease of RCAN-1 mRNA in soleus muscle D group compared with C group ($P<0.05$). No difference was observed in DT group compared with C and D groups ($P>0.05$).

In summary the RCAN-1 as index of calcineurin activation increased in response to resistance training. Effect of calcineurin is showed on growth skeletal muscle in several investigations [7]. Other studies the used this kind of training with ladder demonstrated skeletal muscle hypertrophy in fast twitch muscles [16]. However, in the slow twitch soleus muscle no effect hypertrophic observed with resistance training. The rat soleus, slow-twitch muscle involved in maintaining posture, contains a high percentage of type I fibers [18]. It observed that muscle hypertrophy after heavy resistance training led to fast fibre type conversations [18]. Harris in similar study with ours demonstrated 6 week of resistance training with ladder induced hypertrophy of the mixed fiber type plantaris muscle and not hypertrophic effect on slow twitch soleus muscle [19]. Perhaps soleus muscle activated in this kind of training but fiber type in this muscle did not hypertrophy. In our study RCN-1 mRAN increased with ladder

resistance training in slow twitch skeletal muscle. Expression of RCN-1 is strongly up-regulated in response to calcineurin signaling [12,13]. It seems that with regard to effect of calcineurin on growth skeletal muscle resistance training stimulated calcineurin for skeletal muscle maintain especially in diabetic samples.

Some issues suggested the calcineurin as regulator of skeletal muscle fiber type may has clinical implication for improving insulin action in individuals with diabetes mellitus [20]. Asberg indicated that long-term calcineurin inhibitors treatment negatively effects glucose metabolism [20]. Probably increment in calcineurin activity could be one mechanism for glucose metabolism in our study. Increment in the RCN-1 mRNA had not significant differences with diabetic train group. Despite this we showed significant decrease in calcineurin activity in diabetic control group. Probably resistance training has been able to maintain calcineurin activity in diabetic samples.

The involvement of the calcineurin in the pathogenesis of muscle atrophy is still non-obvious. Muscle wasting induced in rats by spinal cord transection is associated with reduced the the calcineurin protein levels [21], while stimulation of the calcineurin signaling in dystrophic mdx mice reduces muscle damage [22]. Other reports, however, demonstrate that the calcineurin inhibitors neither block the increase in fibre size in regenerating muscle, nor prevent muscle weight recovery induced by intermittent reloading and exercise in two different experimental models of atrophy, namely hindlimb suspension or spinal cord transection [23].

It shown that loss of muscle mass in STZ induced diabetes is also associated with reduced the calcineurin expression and activity. Costelli has demonstrated that the calcineurin is involved in the pathogenesis of muscle wasting in rats with STZ-induced diabetes [24]. The marked muscle atrophy is a characteristic feature of uncontrolled diabetes, as demonstrated in both experimental models [25,26]and in the type1 diabetic patients [27]. Increased proteolysis and inability to repair damaged fibers are both involved in causing muscle depletion [28]. It seems that resistance training is able to maintain the calcineurin signaling in STZ-induced diabetes in our study.

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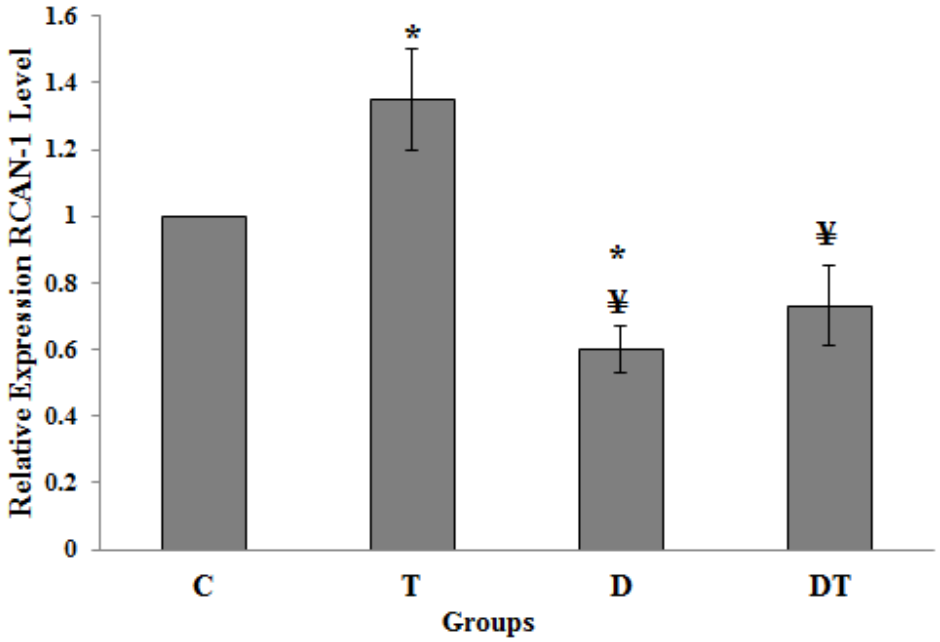

Figure 1: RCAN-1 mRNA expression; All values obtained were normalized to GAPDH and RPL-26 levels. Results were expressed as mean values ± standard error (SE).

**Significantly different from C group (P < 0.05). ¥Significantly different from T group. N = 6–8 animals per group.*

Table 1: Characteristic features of rats in different groups

	C	T	D	DT
Weight	320.3±7.57	317.4±8.66	232.5±10.18*¥	248.3±4.33*¥
Fasting glucose (mmol/l)	4.5±0.57	4.3±0.12	30.4±4.12*¥	27.8±2.41*¥

**Significantly different from C group; ¥ significantly different from T group. N=6-8 animals per group.*

CONCLUSION

In conclusion, we have shown that the RCN-1 mRNA levels as calcineurin activity increased in slow twitch skeletal muscle with resistance training. It seems that resistance training could help to skeletal muscle complications with diabetes.

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