Effect of resistance exercise training on expression of Hsp70 and inflammatory cytokines in skeletal muscle and adipose tissue of STZ-induced diabetic rats

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Abstract Impairment of adipose tissue and skeletal muscles accrued following type 1 diabetes is associated with protein misfolding and loss of adipose mass and skeletal muscle atrophy. Resistance training can maintain muscle mass by changing both inflammatory cytokines and stress factors in adipose tissue and skeletal muscle. The purpose of this study was to determine the effects of a 5-week ladder climbing resistance training program on the expression of Hsp70 and inflammatory cytokines in adipose tissue and fast-twitch flexor hallucis longus (FHL) and slow-twitch soleus muscles in healthy and streptozotocin-induced diabetic rats. Induction of diabetes reduced body mass, while resistance training preserved FHL muscle weight in diabetic rats without any changes in body mass. Diabetes increased Hsp70 protein content in skeletal muscles, adipose tissue, and serum. Hsp70 protein levels were decreased in normal and diabetic rats by resistance training in the FHL, but not soleus muscle. Furthermore, resistance training decreased inflammatory cytokines in FHL skeletal muscle.

On the other hand, Hsp70 and inflammatory cytokine protein levels were increased by training in adipose tissue. Also, significant positive correlations between inflammatory cytokines in adipose tissue and skeletal muscles with Hsp70 protein levels were observed. In conclusion, we found that in diabetic rats, resistance training decreased inflammatory cytokines and Hsp70 protein levels in fast skeletal muscle, increased adipose tissue inflammatory cytokines and Hsp70, and preserved FHL muscle mass. These results suggest that resistance training can maintain skeletal muscle mass in diabetes by changing inflammatory cytokines and stress factors such as Hsp70 in skeletal muscle and adipose tissue.

Keywords Hsp70 · Inflammatory cytokines · Skeletal muscle · Adipose tissue · Resistance training · Type 1 diabetes

Introduction

Type 1 diabetes is an inflammatory autoimmune disease, resulting in a lack of insulin (Cabrera et al. 2016; Price and Tarbell 2015). It has been proposed that impairment of some tissues such as skeletal muscles and adipose tissue accrued following type 1 diabetes is associated with incorrect protein folding (Takada et al. 2007; Ono et al. 2015).

Cells respond to protein-damaging stressors by initiating a response termed the “heat shock” or “stress” response, which involves the rapid and transient increase in a specific set of proteins known as heat shock proteins (Hsp). The major functions of Hsp are protection against apoptotic stimuli and prevention of protein misfolding and aggregation in stress situations (Atalay et al. 2009; Mayer and Bukau 2005). The most highly conserved Hsp protein, Hsp70, has been shown to modulate inflammation in some studies (Borges et al. 2012; Feder and Hofmann 1999; Van Molle et al. 2002). Conversely,

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in certain disease conditions including type 1 diabetes, Hsp70 protein expression is modulated by pro-inflammatory cytokines rather than by metabolic factors (Burkart et al. 2008).

Cytokines appear to be major regulators of adipose tissue metabolism. Therapeutic modulation of cytokine expression offers the possibility of major changes in adipose tissue physiology. Adipocytes can synthesize some inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β) (Coppack 2001; Kern et al. 2001; Wu et al. 2007), and possible relationships between Hsp70 and inflammatory cytokine expression were observed in some studies (Asea et al. 2000; Borges et al. 2013; Van Molle et al. 2002). It was also reported that restraint stress induced Hsp70 in adipose tissue (Yamamoto et al. 2002). Thus, it has been proposed that under stressful conditions, molecular chaperones such as Hsp70 are released as a danger signal to immune cell to promote inflammatory responses and protect cells (Uchida et al. 2012).

Inflammatory cytokines and stress factors may also induce skeletal muscle atrophy in type 1 diabetes (Ono et al. 2015). Resistance exercise training increases the rate of protein synthesis in skeletal muscle (Phillips 2014). Hypertrophy of skeletal muscle and the resulting concomitant gain in power are of great interest for people with disease-induced atrophy such as type 1 diabetes. It has been suggested that aerobic exercise training can change the expression of stress and inflammatory factors in different tissues (Rosa Neto et al. 2009; Lira et al. 2009). On the other hand, some studies proposed that resistance training did not reduce inflammatory and stress factors in individuals with metabolic risk factors (Levinger et al. 2009; Calle and Fernandez. 2010). Thus, the effects of different types of exercise on stress and inflammatory factors in skeletal muscle during type 1 diabetes have not been completely characterized.

At present, no study has examined changes in Hsp70 protein levels in tissues known to be affected by resistance training in type 1 diabetes. Furthermore, the effects of resistance training on inflammatory factors in adipose tissue and skeletal muscle in relation to Hsp70 levels have not been studied. We hypothesized that resistance training would maintain muscle mass and modulate both inflammatory cytokines and stress factors such as Hsp70 in adipose tissue and skeletal muscle of diabetic subjects. In this study, the protein levels of Hsp70 and the inflammatory cytokines IL-6, TNF- α , and IL-1 β in adipose tissue of healthy and streptozotocin (STZ)-induced diabetic rats subjected to resistance exercise training were assessed. Hsp70 protein levels and inflammatory cytokines were also determined in the fast-twitch flexor hallucis longus (FHL) and slow-twitch soleus muscles of normal and diabetic rats subjected to the resistance training targeted to the FHL. Our findings indicate that both diabetes and resistance exercise can change adipose and skeletal muscle Hsp70 levels. Moreover, possible relationships between inflammatory cytokines and Hsp70 in adipose tissue and skeletal muscle were

assessed in the current study. Our findings suggest that changes in inflammatory cytokine and stress factor levels in skeletal muscle and adipose tissue are involved in skeletal muscle adaptation following resistance training in diabetes.

Methods

Animals

Male Wistar rats weighing 250–280 g at the age of 8 to 12 weeks 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 maintained in the Central Animal House, School of Medical Sciences of TMU.

Animals (eight per group) were randomly assigned to the following treatment groups: control (C), trained (T), STZ-induced diabetes (D), and STZ-induced diabetes plus training (DT). Diabetes was induced with IP injection of STZ (Sigma) at 55 mg kg⁻¹ of body weight (BW) in a 0.1 M citrate buffer (pH 4.5). An equal volume of buffer was injected into the control rats. Blood glucose concentrations were assessed after 4 days to ensure that fasting levels greater than 14 mmol l⁻¹ (250 mg dl⁻¹) were reached. Diabetic rats were not treated with insulin during the study, and they showed symptoms of type 1 diabetes, such as polyuria and weight loss.

Resistance training

Rats in the T and DT groups were trained using a ladder-climbing protocol that specifically targets the FHL muscle, with progressively larger weight loads attached to the tail as previously described (Lee et al. 2004). Briefly, animal climbed 26 rungs across the 1-m ladder. One repetition along the ladder required 26 total lifts by the animal (13 lifts per limb). Rats were familiarized with the exercise for 3 days, 48 h before STZ injection, and exercise training was initiated after STZ injection. Rats were positioned at the bottom of the climbing apparatus and motivated to climb the ladder by touching the tail. Exercised animals trained 5 weeks with a rest of 48 h between each exercise session. Animals from the T and DT groups were exercised with five sets of four repetitions each with a 60-s rest interval between the reps and 3 min between the sets per session. At 13 and 14 sessions, rats were decreased to three sets of five repetitions. Weight loads were based on pilot studies for T and ST groups and previous literature reports (Farrell et al. 1999; Molanouri Shamsi et al. 2015) and are provided in Fig. 1 (Molanouri Shamsi et al. 2014). It was not possible to use the same loads relative to body weight for healthy and diabetic rats. Maximal loads that rats could bear prior to training were 120 % of body mass in the DT group and 150 % of body mass in the T group.

Fig. 1 Protocol for resistance training using ladder climbing. Amount of weight lifted by diabetic ($N=8$) and normal ($N=8$) rats during 17 sessions of resistance exercise

Tissue and serum collection

Twenty-four hours after the last training session, rats were anesthetized with a mixture of Ketamine™ (30–50 mg kg⁻¹ BW, IP) and Xylazine (3–5 mg kg⁻¹ BW, IP). The soleus and FHL muscles and flank subcutaneous adipose tissue were quickly extracted and stored in liquid nitrogen for subsequent analysis. For the serum samples, at least 5 ml blood were taken by intracardiac puncture from each rat, followed by euthanasia using cervical dislocation. Blood samples were centrifuged for 10 min at 4000 rpm and serum was stored at -70 °C.

Muscle and adipose tissue preparation

Frozen tissues (0.1–0.3 g) were homogenized in RIPA buffer (0.625 % Nonidet P-40, 0.625 % sodium deoxycholate, 6.25 mM sodium phosphate, and 1 mM EDTA at pH 7.4) containing 10 µg ml⁻¹ of a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Homogenates were centrifuged at 12,000g for 10 min at 4 °C, the supernatant was saved, and protein concentrations were determined using the Bradford assay (Bio-Rad, Hercules, CA) with bovine serum albumin as a reference (Bradford 1976). Supernatants were aliquotted and stored at -80 °C.

Assay of cytokines and Hsp70

TNF-α, IL-6, IL-1β, and Hsp70 protein levels in tissue and serum were determined in duplicate using commercially available rat ELISA kits (DuoSet ELISA, R&D Systems, Minneapolis, MN). The assays were carried out according to the manufacturer's instructions. Data are expressed as picogram of cytokine per milligram of total protein. The minimum detectable concentrations were <5 pg ml⁻¹ for IL-1β and TNF-α, <20 pg ml⁻¹ for IL-6, and <50 pg ml⁻¹ for Hsp70. The intra- and inter-assay coefficients of variation were 2.7 and 5.2 % for TNF-α, 4.9 and 9.5 % for IL-6, 5.5 and 4.7 % for IL-1β, and 10 and 16 % for Hsp70.

Blood glucose and serum insulin measurements

Fasting blood was sampled from the tail vein 24 h after the last exercise session, following an overnight fast. Blood glucose levels were tested by glucometer (GT-1920, Japan) with samples run in duplicate. Serum insulin concentrations were measured using a commercially available ultrasensitive rat insulin ELISA kit (ALPCO Diagnostics, Windham, NH).

Statistical analysis

All analyses were performed using SPSS V16.0 (SPSS, Chicago, IL). Two-way analysis of variance (two-way ANOVA) and Tukey post hoc tests were used for protein data. Also, two-way ANOVA was used for the characteristic features of rats in different groups. Furthermore, independent *t* tests were used to detect differences between FHL and soleus muscles. Correlation analyses were performed using Pearson's test. Statistical significance was set at $P<0.05$. Data are presented as means ± SEM.

Results

Induction of diabetes mellitus and effects of training on muscle mass

Rats underwent the protocols for control (C), trained (T), STZ-induced diabetes (D), and diabetes with training (DT) as outlined in the “Methods”. The characteristic features of the rats in different groups are presented in Table 1, which shows that the diabetic groups displayed significant reductions in plasma insulin levels compared to the healthy groups. Consequently, typical type 1 diabetes hyperglycemia occurred in D and DT groups, with increased fasting glucose. Moreover, at the end of the study, diabetic rats showed a significant decrease in total BW, although different groups did not have noticeable differences in total weight at the beginning of the study (Table 1). Five weeks of resistance training resulted in hypertrophy of the fast FHL muscle in the T and DT groups, as indicated by a significantly higher ratio of FHL weight-to-body weight in trained compared to sedentary animals (Table 1). Although total BW diminished in the both D and DT groups, the ratio of FHL muscle to body weight was significantly higher in the DT group compared with the D group. However, no significant differences in muscle-to-body weight ratio were observed for the primarily slow soleus muscle in either T or DT group (Table 1). These data are consistent with previous studies indicating preferential hypertrophy of the fast FHL muscle in this ladder climbing resistance exercise protocol (Lee et al. 2004; Hornberger and Farrar 2004; Molanouri Shamsi et al. 2015).

Table 1 Characteristic features of rats in different groups

	C	T	D	DT
Initial weight (g)	266.9 ± 7.09 <i>a</i>	260.38 ± 5.34 <i>a</i>	262.5 ± 4.86 <i>a</i>	261.75 ± 9.37 <i>a</i>
Final weight (g)	320.3 ± 7.57 <i>a</i>	317.38 ± 8.66 <i>a</i>	232.51 ± 10.18 <i>b</i>	248.3 ± 4.33 <i>b</i>
Fasting glucose (mmol l ⁻¹)	4.52 ± 0.57 <i>a</i>	4.34 ± 0.12 <i>a</i>	30.37 ± 4.12 <i>b</i>	27.76 ± 2.41 <i>b</i>
Fasting insulin (ng ml ⁻¹)	0.61 ± 0.04 <i>a</i>	0.72 ± 0.05 <i>a</i>	0.14 ± 0.03 <i>b</i>	0.21 ± 0.02 <i>b</i>
FHL muscle mass (mg)	576.6 ± 19.64 <i>a</i>	646.62 ± 8.88 <i>b</i>	416.6 ± 11.93 <i>c</i>	494.5 ± 3.8 <i>d</i>
FHL-to-body mass ratio (mg g ⁻¹ × 100)	179.61 ± 2.17 <i>a</i>	204.6 ± 5.13 <i>b</i>	175.4 ± 9.83 <i>a</i>	199.64 ± 2.19 <i>b</i>
Soleus muscle mass (mg)	160.4 ± 14.34 <i>a</i>	164.7 ± 11.17 <i>a</i>	110.4 ± 3.02 <i>b</i>	123.14 ± 4.32 <i>b</i>
Soleus-to-body mass ratio (mg g ⁻¹ × 100)	49.61 ± 3.71 <i>a</i>	51.96 ± 9.34 <i>a</i>	46.06 ± 1.1 <i>a</i>	49.65 ± 1.48 <i>a</i>

Data were analyzed by two-way ANOVA; for each parameter, values with different italicized letters differ significantly at $P < 0.05$. $N = 8$ animals per group

Hsp70 protein levels in skeletal muscle, adipose tissue, and serum

Adipose tissue Hsp70 protein levels of diabetic groups were significantly higher than those of the normal groups (Fig. 2a). Additionally, a significant training × diabetes interaction was also observed for the adipose tissue. Post hoc analyses indicated that Hsp70 protein expression increased in all groups compared with the C group (Fig. 2a). Also, there is a significant difference between control diabetic and trained diabetic animals (Fig. 2a). In the soleus muscle, a significant effect of diabetes on Hsp70 protein expression was observed (Fig. 2b). However, in the FHL muscle, Hsp70 protein

expression exhibited a significant effect of training (Fig. 2c). Resistance training decreased Hsp70 protein in FHL muscle of diabetic rats about 45 % compared to control diabetic rats. This was about 20 % in the soleus muscle (Fig. 2b and c). Also, there are significant differences between slow- and fast-twitch skeletal muscles in each group for Hsp70 protein expression (t test result, not shown; $P < 0.01$). Hsp70 protein expression was significantly higher in the soleus muscle compared with FHL skeletal muscle in all groups (Fig. 2b and c). Also, serum Hsp70 concentrations were significantly higher in the diabetic groups compared the normal groups (Fig. 2d). Resistance exercise training did not change serum Hsp70 concentrations.

Fig. 2 Effects of resistance training on Hsp70 protein expression in normal and diabetic rats. **a** Hsp70 protein expression in adipose tissue. **b**, **c** Hsp70 protein expression in soleus (**b**) and flexor hallucis longus (FHL; **c**). **d** Serum Hsp70 levels in picogram per milliliter. Groups are C healthy control, sedentary rats; T trained healthy rats; D diabetic sedentary

rats; DT diabetic trained rats. Results are expressed as mean ± standard error (SE). Results from the two-way ANOVA are presented. For each panel, bars with different superscripts are significantly different ($P < 0.05$). *Differ from C group, #Differ from D group. $N = 6-8$ animals per group

Inflammatory cytokine levels in adipose tissue and skeletal muscle

The effects of diabetes and resistance training on protein levels of IL-6, TNF- α , and IL-1 β in the FHL and soleus muscles were assessed. Significantly higher concentrations of IL-6 and IL-1 β were observed in diabetic FHL and soleus muscle compared to healthy rats. Training decreased muscle IL-6 levels in both the soleus and FHL muscles of diabetic rats (training $\times$ diabetes interaction, $P < 0.001$). No training-dependent changes were observed for the other cytokines (Molanouri Shamsi et al. 2014).

Figure 3 shows the effects of diabetes and resistance training on protein levels of IL-6, TNF- α , and IL-1 β in adipose tissue. Significantly higher concentrations of inflammatory cytokines were observed in diabetic adipose tissue compared to healthy rats (Fig. 3a, b, and c). Resistance training increased IL-6 and TNF- α protein levels in adipose tissue (Fig. 3a and c). Additionally, a significant training $\times$ diabetes interaction was observed for TNF- α protein levels in adipose tissue (Fig. 3c). Post hoc analyses indicated that TNF- α protein levels increased in all groups compared with the C group. Training did not affect adipose tissue IL-1 β protein levels (Fig. 3b).

Correlations between cytokine concentrations with Hsp70 protein levels in adipose tissue and skeletal muscles

Significant positive correlations between Hsp70 protein levels and IL-1 β and TNF- α concentrations in FHL skeletal muscle were observed (Table 2). Also, in soleus skeletal muscle, significant positive correlations were observed between Hsp70 levels and IL-6 and TNF- α concentrations (Table 2). Furthermore, significant positive correlations were found between inflammatory cytokines and Hsp70 protein levels in adipose tissue (Table 2).

Discussion

This study was undertaken to test the hypothesis that resistance training would maintain muscle mass, as well as change inflammatory cytokine and Hsp70 levels in adipose tissue and skeletal muscle in diabetic subjects. Additionally, possible relationships between inflammatory cytokine and Hsp70 levels in adipose tissue and skeletal muscle were investigated. Also, we determined if skeletal muscle fiber type could affect Hsp70 expression in response to a resistance training regimen targeted to one type of muscle. The results of this study showed that diabetes decreased both soleus and FHL muscle

Fig. 3 Cytokine concentrations in adipose tissue from the four groups of rats. **a** IL-6 concentrations in adipose tissue; two-way ANOVAs revealed significant effects of diabetes and training for IL-6. **b** IL-1 β concentrations in adipose tissue; two-way ANOVAs revealed significant effect of diabetes for IL-1 β in adipose tissue. **c** TNF- α concentrations in adipose tissue; two-way ANOVAs revealed significant effects of

training $\times$ diabetes interactions for TNF- α in adipose tissue. For all panels, bars with different superscripts are significantly different ($P < 0.05$). *Differ from C group. Groups are C healthy control, sedentary rats; T trained healthy rats; D diabetic sedentary rats; DT diabetic trained rats. Results are expressed as mean $\pm$ standard error (SE)

Table 2 Correlations between cytokine concentrations in adipose tissue and skeletal muscles with Hsp70 protein levels

	FHL muscle	IL-6	IL-1 β	TNF- α	Soleus muscle	IL-6	IL-1 β	TNF- α	Adipose tissue	IL-6	IL-1 β	TNF- α
Hsp70 protein		0.62 ^a	0.39	0.59 ^a		0.29	0.49 ^a	0.48 ^a		0.55 ^a	0.73 ^b	0.72 ^b

Significant correlations (^a $P < 0.05$, ^b $P < 0.001$), obtained using Pearson's product-moment correlation

mass, whereas FHL skeletal muscle weight increased following resistance training in both healthy and diabetic subjects, with no effect on soleus muscle weight. While diabetes decreased body mass, we did not observe any differences between control and training groups in body mass in either healthy or diabetic rats. Hsp70 protein expression exhibited divergent changes in skeletal muscles and adipose tissue following resistance training. Hsp70 protein levels increased following resistance training in adipose tissue. On the other hand, Hsp70 protein expression was decreased in fast-twitch skeletal muscle following resistance training. Moreover, serum Hsp70 protein levels increased in diabetic rats, with no effect of resistance training. STZ-induced diabetes and resistance training increased the levels of the inflammatory cytokines IL-6 and TNF- α in adipose tissue. On the other hand, resistance training decreased IL-6 expression in FHL skeletal muscle. Positive significant correlations were found between levels of these inflammatory cytokines in adipose tissue and skeletal muscles with Hsp70 protein levels.

STZ-induced diabetes and resistance training increased the expression of inflammatory cytokines and Hsp70 in adipose tissue. STZ-induced diabetes mellitus has been used to study the pathophysiology of diabetes and may resemble the natural course of type 1 diabetes mellitus in humans (Takada et al. 2007; Portha et al. 1989). This rodent model exhibits several metabolic derangements, an impairment of adipose tissue and skeletal muscle, loss of adipose mass, and skeletal muscle atrophy that are similar to the human disorder (Coleman et al. 2015; Ono et al. 2015). In the current study, we did not observe any differences in overall body mass between trained and untrained diabetic animals. But, there was a significant increase in FHL, but not soleus, muscle mass in trained animals. Thus, resistance exercise training decreased adipose tissue and increased mass of the muscle type targeted by the exercise regimen. In situations such as uncontrolled type 1 diabetes, energy supplies such as adipose tissue are very important, and it is possible that decreases in adipose tissue mass could directly (via lipolysis) and indirectly (via release of stress factors and cytokines) aid in maintenance or hypertrophy of skeletal muscle under appropriate stimuli such as exercise (Gunawardana 2012; Coleman et al. 2015).

On the other hand, adipose tissue is not only a storage organ for excess calories, but also produces some hormone-like factors such as cytokines and stress factors (Gomez-

Merino et al. 2007; You et al. 2005). These factors are thought to have both local and systemic metabolic actions (You et al. 2005; Lau et al. 2005). Moreover, type 1 diabetes is an inflammatory autoimmune disease, resulting in a lack of insulin. When the immune system is challenged, an important flow of energy is required. The metabolic potential of adipose tissue and the energy needs of the immune system have probably led to the evolutionary development of feedback relationships between the two systems (Batra and Siegmund 2012; Lago et al. 2007; Speaker et al. 2014).

Intracellular Hsp functions by binding non-covalently to misfolded, aggregated, and nascent proteins and either assist in their proper folding, solubility, and appropriate translocation, or alternatively, eliminate them through the degradation pathway (Westerheide and Morimoto 2005). It is possible that increased Hsp70 protein expression in adipose tissue following resistance training could help this tissue to maintain structure, or reflect a response to stress mediated by inflammatory cytokines or apoptosis. Positive correlations between Hsp70 and inflammatory cytokines in adipose tissue can approve this point.

The constant stress to skeletal muscle tissue associated with diabetes could elevate constitutive expression of Hsp70 in skeletal muscles. Additionally, as confirmed in this study, constitutive expression of Hsp70 was higher in slow muscles compared to fast muscles (O'Neill et al. 2006; Locke et al. 1991). Some studies concluded that the basis of fiber-specific differences in Hsp70 expression are due to slow muscles being more frequently recruited for ambulation and postural maintenance, resulting in increased fiber damage, protein turnover, oxidative stress, and temperature associated with frequent contractile activity (O'Neill et al. 2006; Locke et al. 1991; Ogata et al. 2005). Resistance training could not change basal levels of Hsp70 in soleus muscle. Frier and Locke (2007) suggested consequent accumulation of Hsps may inhibit increases in muscle mass and total muscle protein content in muscles undergoing hypertrophy (Frier and Locke 2007). Also, it has been proposed that basal content of Hsps in skeletal muscle is negatively related to their changes following exercise and training. It seems that higher basal levels of Hsp in Soleus skeletal muscle could be a reason for no changes following exercise training (Gjøvaag et al. 2006; Morton et al. 2009; Paulsen et al. 2012). On the other hand, studies have shown that type II (fast) fibers undergo the most

severe atrophy due to hyperglycemia (Russell et al. 2009; Wang and Pessin 2013), but are also more responsive to hypertrophic stimuli (Fitts and Widrick 1996). Loss of fast isomyosins and appearance of slow isoforms have been demonstrated in skeletal muscles of rats following 4 weeks of STZ injection (Rutschmann et al. 1984). It is probable that STZ-induced diabetes could stimulate HSP70 expression in by both induction of stress and a fiber-type shift. Hsp70 protein levels were decreased in the FHL muscle following resistance training, an effect which could likewise be due to a combination of mechanisms.

In this regard, our findings concerning the effects of the combination of diabetes and training on Hsp70 levels in skeletal muscle contribute novel information to the literature on stress-related proteins and these conditions. As mentioned above, exercise-induced stress and muscle damage are considered two out of many stimuli, which induce Hsp synthesis in skeletal muscle (Steinacker et al. 2004). Single bouts of exercise increased levels of Hsp, especially, Hsp70 protein in skeletal muscle in healthy subjects (Fischer et al. 2006; Morton et al. 2006; Tupling et al. 2007). Likewise, high-intensity endurance or resistance exercise training has been shown to upregulate the muscular Hsp70 in athletes (Liu et al. 2004; Vogt et al. 2001). Our study demonstrated significant hypertrophy of the FHL, but not soleus skeletal muscle in both healthy and diabetic rats. The applied resistance training in the present study was ladder climbing, which has been found to produce selective hypertrophy of the FHL muscle due to its eccentric mode of action during this activity (Lee et al. 2004; Molanouri Shamsi et al. 2015; Hornberger and Farrar 2004). This was associated with decreases in Hsp70 levels in the FHL in response to exercise in both healthy and diabetic rats that were not observed in the soleus, which was not targeted by this training protocol. The process of muscle hypertrophy has been suggested to be preceded by muscle-damaging stress (Bischoff and Heintz 1994), indicating that an acute exercise overload may be considered stressful and may initiate the stress responses and production of inflammatory mediators (Malm 2001; Noble and Shen 2012; Silver et al. 2012). However, some studies have shown that exercise training adaptations instead decreased inflammatory mediators and stress factors in skeletal muscle (Atalay et al. 2004; Calle and Fernandez 2010; Molanouri Shamsi et al. 2014). Both animal and human studies have suggested that stress factors and inflammatory mediators play important roles in skeletal muscle wasting in type 1 diabetes (Coleman et al. 2015; Krause et al. 2011). In our study, decreased IL-6 and Hsp70 expression were observed in the FHL muscle in diabetic rats. Because our training intervention resulted in FHL muscle hypertrophy, our findings suggest that resistance training may attenuate skeletal muscle wasting by suppressing inflammation and stress factors.

The present study showed a significant increase in serum Hsp70 concentrations due to diabetes. Resistance exercise training did not change serum Hsp70 concentrations. There are other reports of Hsp70 serum levels in type 1 diabetic samples (Gruden et al. 2009; Oglesbee et al. 2005; Nakhjavani et al. 2010). Oxidative stress and inflammation contribute to chronic complications of diabetes (Wei et al. 2009). Inflammatory cytokines, ischaemia and free oxygen radicals are able to induce Hsp70 expression (Najemnikova et al. 2007). However, the source of circulating Hsp in healthy individuals, as well as in patients with pathological conditions has not been identified yet. Hsp can be released into the circulation and exert an immune stimulatory effect by interacting with pattern recognition receptors, such as toll-like receptors, and thereby activate the host inflammatory response (Asea et al. 2002; Johnson et al. 2006). Aerobic exercise training has been shown to decrease markers of inflammation in plasma (Mardare et al. 2016). Despite this, even long duration resistance training could not decrease inflammation in some studies (Hansen et al. 2011; Lee et al. 2011). It seems that for circulatory immunological adaptation following exercise training, long-term concurrent resistance and aerobic exercise training can be considered.

In summary, FHL skeletal muscle mass was increased following resistance training. On the other hand, Hsp70 protein was decreased in fast-twitch skeletal muscle after resistance training. Also, we observed higher Hsp70 protein levels in slow-twitch skeletal muscle compared to fast-twitch skeletal muscle. Diabetes and resistance training increased Hsp70 and inflammatory cytokine levels in adipose tissue. On the other hand, resistance training decreased Hsp70 and IL-6 protein levels in fast-twitch skeletal muscle. Our study demonstrates positive correlations between Hsp70 and inflammatory cytokine levels in adipose tissue and skeletal muscles. Our results propose that resistance training maintain the skeletal muscle mass in diabetes by changing inflammatory cytokines and stress factors such as Hsp70 in skeletal muscle and adipose tissue. Further studies are in order to determine the mechanism controlling Hsp70 and inflammatory cytokines in adipose tissue and skeletal muscle following resistance training.

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Compliance with ethical standards All experiments involving animals were conducted according to the policies of the 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 Medicine Sciences, Tarbiat Modares University (TMU), Tehran, Iran.

Conflict of interest The authors declare that they have no conflict of interest.

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