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Effect of Resistance Exercise Training Associated with Skeletal Muscle Hypertrophy on Serum Pro-Inflammatory Cytokines in STZ-induced Diabetes

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ABSTRACT

Skeletal muscle atrophy is associated with type 1 diabetes. Effects of resistance exercise training associated with skeletal muscle hypertrophy on serum inflammatory cytokines was exactly not clarified. Protein levels of inflammatory cytokines IL-6, TNF-α, and interleukin-1beta (IL-1β) in serum of healthy and streptozotocin (STZ)-induced diabetic rats subjected to resistance exercise training were assessed in this study. Rats were divided into the control, training, control diabetic and diabetic training groups. Training groups performed the resistance training consisted of climbing a 1 m ladder with increasing weight added to the tail. Proteins levels of IL-6, TNF-α and IL-1β in serum were measured by the ELIZA method. The results of this study indicated that resistance training induced skeletal muscle hypertrophy in diabetic samples ($P<0.05$). Also, Resistance training decrease IL-6 protein levels in serum. Inflammatory cytokines could act as stress factors in diabetes. It seems that this kind of exercise training individually could not change cytokines levels in serum.

Key words: Inflammatory cytokines, Resistance training, Type-1diabetes

INTRODUCTION

Type 1 diabetes is an inflammatory autoimmune disease, resulting in a lack of insulin and hyperglycemia [1,2]. The molecular mechanisms underlying hyperglycemia induced effects on inflammation and vascular complications are thought to involve the action of reactive oxygen species within the cell nucleus [3]. The underlying rationale for our study was that diabetes, and the concomitant oxidative stress associated, may induce an inflammatory response. It is widely accepted that the levels of inflammatory cytokines increase under stressful conditions [4,5,6].

Accumulating evidence supports recommending regular physical activity to prevent and treat diabetes and otherchronic diseases that present a constant pro-inflammatory status[7]. Regular physical activity is known to bring health benefits, such as increased insulin sensitivity, glycemic control, decrease of body weight and percentage of body fat, lower blood pressure, and reduction of overall risk of vascular disease [8,9].The immune-modulatory effects of regular exercise, and in particular, resistance training, may have positive effects on innate immunity and so may provide benefits for the profile of diabetes in addition to improving strength and functional abilities [10]. Such effects have been considered dependent on contractile muscle activity through IL-6 production, a cytokine that exerts inhibitory effects on several pro-inflammatory cytokines, including TNF-a [11]. Unlike studies in obesity and type 2 diabetes with insulin resistance, very little is known about the expression patterns inflammatory cytokines in type 1 diabetic models following exercise training.

Resistance exercise training increases the rate of protein synthesis in skeletal muscle [12]. Also, protective effects of resistance exercise training on diabetic situations were observed in several studies [13,14].On the other hand,

hypertrophy of skeletal muscle and the resulting concomitant gain in power are of great interest for people with disease-induced atrophy. Increased expression of inflammatory cytokines has been identified as one of the possible mechanisms of muscle atrophy in diabetes [15]. Aerobic exercise training has been shown to decrease the production of pro-inflammatory cytokines in plasma and tissues [16]. But effects of resistance exercise training on inflammatory cytokines were not exactly clarified.

As we know effect of resistance training associated with skeletal muscle hypertrophy on inflammatory cytokines in type-1 diabetes have not been assessed. We hypothesized that diabetes would modulate the effects of resistance exercise on inflammatory cytokines in serum. In this study, protein levels of inflammatory cytokines IL-6, TNF-α, and interleukin-1beta (IL-1β) in serum of healthy and streptozotocin (STZ)-induced diabetic rats subjected to resistance exercise training were assessed.

MATERIALS AND METHODS

Animal

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. 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 maintained in the Central Animal House, School of Medical Sciences of TMU.

Animals (8 per group) were randomly assigned to the following the 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 [17]. 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 three days, 48 hours before STZ injection, and exercise training was initiated after STZ injection. Rats were positioned at the bottom of 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.

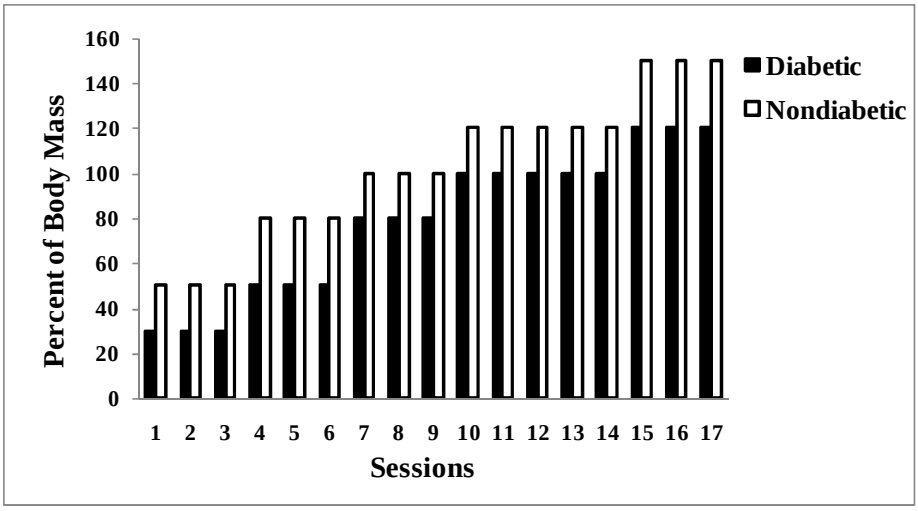

Figure1. Protocol for resistance training using ladder climbing
Amount of weight lifted by diabetic (N=8) and normal (N=8) rats during 17 sessions of resistance exercise.

Animals from the T and DT groups were exercised with 5 sets of 4 repetitions each with a 60 sec rest interval between the reps and 3 min between the sets per session. At 13 and 14 sessions, rats were decreased to 3 sets of 5 repetitions. Weight loads were based on pilot studies for T and ST groups and previous literature reports [14,18]and

are provided in Fig. 1[13]. 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 DT group and 150% of body mass in T group.

Serum collection

Twenty-four hours after the last training session, rats were anesthetized with a mixture of Ketamine^{TM} (30–50 mg.kg^{-1} BW, IP) and Xylazine (3–5 mg.kg^{-1} BW, IP). Serum samples, at least 5 ml blood were taken by intra cardiac puncture from each rat, followed by euthanasia using cervical dislocation. Blood samples were centrifuged for 10 minutes at 4000 rpm and serum stored at -70^{o}C.

Assay of cytokines

TNF-α, IL-6 and IL-1β protein levels in 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 manufacturers' instructions. Data are expressed as pg of cytokine per ml. The minimum detectable concentrations were <5 pg.ml^{-1} for IL-1β and TNF-α and <20 pg.ml^{-1} for IL-6. 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.

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 were used for characteristic features of rats in different groups. 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 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[13]. These data are consistent with previous studies indicating preferential hypertrophy of the fast FHL muscle in this ladder climbing resistance exercise protocol [17,19].

Table 1. Characteristic features of rats in different groups

	C	T	D	DT
Initial weight (g)	266.9±7.09 *a*	260.38±5.34 *a*	262.5±4.86 *a*	261.75±9.37 *a*
Final Weight (g)	320.3±7.57 *a*	317.38±8.66 *a*	232.51±10.18 *b*	248.3±4.33 *b*
Fasting glucose (mmol.l^{-1})	4.52±0.57 *a*	4.34±0.12 *a*	30.37±4.12 *b*	27.76±2.41 *b*
Fasting insulin (ng.ml^{-1})	0.61±0.04 *a*	0.72±0.05 *a*	0.14±0.03 *b*	0.21±0.02 *b*
FHL muscle mass (mg)	576.6±19.64 *a*	646.62±8.88 *b*	416.6±11.93 *c*	494.5±3.8 *d*
FHL-to-body mass ratio (mg.g^{-1}×100)	179.61±2.17 *a*	204.6 ±5.13 *b*	175.4 ±9.83 *a*	199.64 ±2.19*b*

Data were analyzed by 2-way ANOVA; for each parameter, values with different italicized letters differ significantly at P < 0.05. N=8 animals per group.

Inflammatory cytokine levels in Serum

Fig 2 shows the effects of diabetes and resistance training on protein levels of IL-6, TNF-α, and IL-1β in serum. We did not observe significantly higher concentrations of inflammatory cytokines in diabetic serum compared to healthy rats (Fig 2 A, B, C). Resistance training decreased IL-6 protein levels in serum (Fig 2 A). on the other hand, training did not affect serum TNF-α and IL-1β serum protein levels (Fig 2B,C).

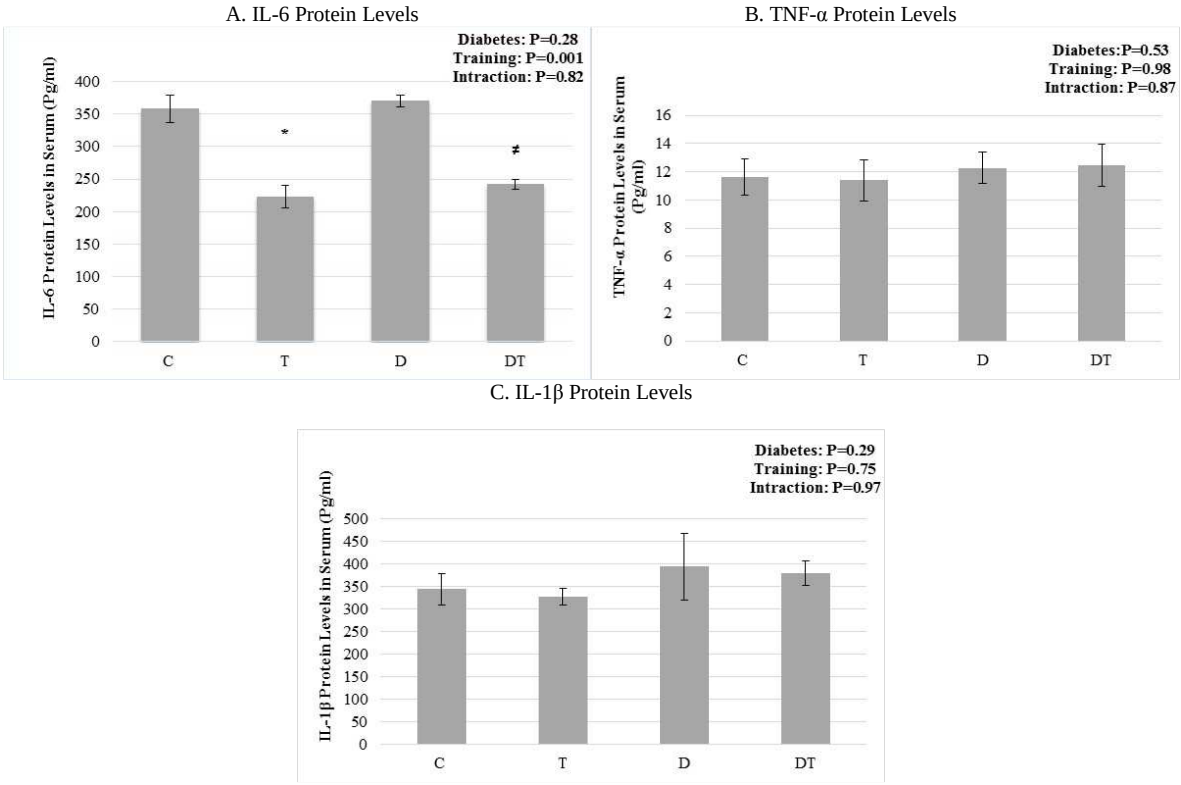

Figure 2. Cytokine concentrations in serum from the four groups of rats

A: IL-6 concentrations in serum; two-way ANOVAs revealed significant effects of training for IL-6. B: TNF-α concentrations in serum; two-way ANOVAs did not reveal significant effects of training and diabetes for TNF-α in serum. C: IL-1B concentrations in serum; two-way ANOVAs did not reveal significant effect of diabetes and training for IL-1B in serum. For all panels, bars with different superscripts are significantly different ($P<0.05$). * differ from C group, ≠ differ from D group.Groups are: C healthy control, sedentary rats; T, trained healthy rats; D, diabetic sedentary rats; DT, diabetic trained rats. N = 6–8 animals per group. Results are expressed as mean ± standard error (SE).

DISCUSSION

This study was undertaken to test the hypothesis that resistance training would maintain muscle mass, as well as change inflammatory cytokine in serum in diabetic subjects. The results of this study showed that decreased FHL muscle mass, whereas FHL skeletal muscle weight increased following resistance training in both healthy and diabetic subjects. 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. STZ-induced diabetes did not change serum inflammatory cytokines.On the other hand, resistance training decreased IL-6 serum protein levels in diabetic and normal rats.

The applied resistance training in the present study was ladder climbing. Ladder climbing has been found to produce increases in the mass of the FHL muscle, while the mass of other muscle groups was not affected[18,19,20]. It seems that in this kind of resistance training various training-related variables such as frequency, duration, sets, intensity and repetition of exercise sessions are important for adaptation in different muscles [21]. Hornberger and Farrar (2004) observed the FHL muscle hypertrophy following progressive resistance exercise with ladder in the rat [19]. Also, after 6 weeks' resistance training increase in muscle mass in the FHL in the young and aged animal was observed[20]. Lee et al (2004) suggested that selective hypertrophy of FHL muscle is due to its eccentric mode of action during climbing. It is shown that muscle hypertrophy after heavy resistance training only observe in fast type fiber[17].In a similar study, Harris et al (2009) demonstrated that resistance training with ladder induces hypertrophy of the mixed fiber type plantaris muscle and do not have hypertrophic effect on slow twitch soleus muscle [20].

We did not observe any changes in TNF-α and IL-1β following resistance training. Several studies have shown that exercise training leads to an increase of serum levels of anti-inflammatory cytokine[22,23]. Based on its anti-inflammatory effects, exercise can also be used as a means to control low-grade systemic inflammation [11]. Also, exercise training could diminish the skeletal muscle wasting in diabetic rats by decreasing oxidative stress and

inhibiting muscle-specific ring-finger protein 1 (MuRF1) expression at both the mRNA and protein levels [24]. On the other hand, inappropriate exercise intensity can worsen this dysregulation, contributing to the metabolic, inflammatory, and stress disorders associated with metabolic syndrome[25]. In addition, resistance training involving eccentric actions induces muscular damage to a higher extent than concentric actions [26]. Numerous studies have shown that eccentric exercise-induced muscle damage triggers inflammatory responses characterized by releases of leukocytes and cytokines[27]. Cytokine response may vary by the type of exercise, intensity, duration, recovery between exercise bouts, and training status [28].

In our study inflammatory cytokines were not changed after resistance training. To date, the findings of studies that examined the effect of resistance training alone on pro-inflammatory markers in human or animals with diabetes mellitus are limited and inconsistent[29,30]. Brooks et al. (2006) reported that16 weeks of resistance training in elderly individuals with type 2 diabetes reduced plasma CRP concentration compared with the control group[31]. Similarly, Olson et al. (2007)reported that long-term resistance training (1 year, 2 days/week) significantly reduced plasma CRP concentration compared with baseline levels in overweight women[32]. In contrast, Levinger et al. (2009) indicated that resistance training as a single intervention did not modify any inflammatory marker at rest for any of the tested groups. Authors suggested that long-term resistance training interventions may be required to see the effect on the tested inflammatory markers [33].

Aerobic exercise training has been shown to decrease the production of pro-inflammatory cytokines in plasma and tissues. Despite this, even long duration resistance training could not decrease inflammation [34,35]. The importance of inflammatory events in promoting or preventing skeletal muscle hypertrophy is unclear. With attention to previous studies, it seems that inflammation is related to adaptations induced with resistance training[34,35,36]. Therefore, combining resistance and aerobic training could be an effective way to prevent and delay muscle inflammatory events induced with resistance training especially for inflammatory cytokines related to diseases in skeletal muscle.

CONCLUSION

In summary, in our study only IL-6 protein levels decreased in response to training in diabetes, suggesting that for desired changes in other inflammatory cytokines, aerobic or concurrent training can be considered. Our study showed that resistance exercise exhibited positive effects on muscle mass in both healthy and diabetic rats.

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REFERENCES

[1]Cabrera SM, HenschelAM, Hessner MJ. Innate inflammation in type 1 diabetes.Transl Res. 2016; 167(1):214-27.
[2]Price JD, Tarbell KV. The Role of Dendritic Cell Subsets and Innate Immunity in the Pathogenesis of Type1DiabetesandOtherAutoimmune Diseases. FrontImmunol. 2015;15(6):288.
[3] Wright E Jr, Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. Int J ClinPract. 2006; 60:308–314
[4] Ireland HE, Leoni F, Altaie O, Birch CS, Coleman RC, Hunter-Lavin C et al. Measuring the secretion of heat shock proteins from cells. Methods. 2007; 43:176–183
[5]Pandey KB, Mishra N, Rizvi SI. Protein oxidation biomarkers in plasma of type 2 diabetic patients. ClinBiochem. 2009; 23:23
[6]Soti C, Nagy E, Giricz Z, Vigh L, Csermely P, Ferdinandy P. Heat shock proteins as emerging therapeutic targets. Br J Pharmacol. 2005; 146:769–780
[7] Pedersen BK. The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control. Essays Biochem. 2006; 42, 105–117
[8] Caspersen CJ, Fulton JE, Epidemiology of walking and type 2 diabetes. Med Sci Sports Exerc. 2008; 40, 519–528
[9]Wilund KR. Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease? ClinSci (Lond). 2008; 112, 543–555
[10] Li ZZ, Liu JB, Jiao L, Chen L. Intensive therapy for diabetes through influence on innate immune system.Med Hypotheses. 2009;72, 675–676
[11]Mathur N, Pedersen BK. Exercise as a mean to control low grade systemic inflammation. Mediators Inflamm. 2008; 2008:109502

[12]Phillips SM. A brief review of critical processes in exercise-induced muscular hypertrophy. Sports Med. 2014; 44,1:S71-7.
[13]MolanouriShamsi M, Hassan ZH, Gharakhanlou R, Quinn LS, Azadmanesh K, Baghersad L et al. Expression of interleukin-15 and inflammatory cytokines in skeletal muscles of STZ-induced diabetic rats: effect of resistance exercise training.Endocrine. 2014; 46(1):60-9.
[14]MolanouriShamsi M, Hassan ZM, Quinn LS, Gharakhanlou R, Baghersad L, Mahdavi M. Time course of IL-15 expression after acute resistance exercise in trained rats: effect of diabetes and skeletal muscle phenotype.Endocrine. 2015; 49(2):396-403.
[15]Hunter RB, Stevenson E, Koncarevic A, Mitchell-Felton H, Essig DA, Kandarian SC. Activation of an alternative NF-kappaB pathway in skeletal muscle during disuse atrophy. FASEB J. 2002;16(6):529-38.
[16]Bruce CR, Hawley JA. Improvements in insulin resistance with aerobic exercise training: a lipocentric approach. Med Sci Sports Exerc. 2004;36(7):1196-201.
[17]Lee S, Barton ER, Sweeney HL, Farrar RP.Viral expression of insulin-likegrowthfactor-Ienhancesmuscle hypertrophy in resistance-trained rats.JAppl Physiol.2004;96(3):1097-104.
[18]FarrellPA, Fedele MJ, Hernandez J, Fluckey JD, Miller JL 3rd, Lang CH et al. Hypertrophy of skeletalmuscle in diabetic rats in response to chronic resistance exercise.J. Appl Physiol. 1999;87(3):1075-82.
[19]Hornberger TA Jr, Farrar RP. Physiological hypertrophy of the FHL muscle following 8week sofprogressive resistance exercise in the rat.Can J Appl Physiol. 2004; 29(1):16-31.
[20]Harris MB, Slack KN, Prestosa DT, Hryvniak DJ. Resistance training improves femoral artery endothelial dysfunction in aged rats.Eur J Appl Physiol. 2010;108(3):533-40.
[21]Klitgaard H. A model for quantitative strength training of hindlimb muscles of the rat.J Appl Physiol. 1988; 64(4):1740-5.
[22]Puglisi MJ, Fernandez ML. Modulation of C-reactive protein, tumor necrosis factor-alpha, and adiponectin by diet, exercise, and weight loss.J Nutr. 2008;138(12):2293-6.
[23]Kadoglou NP, Iliadis F, Angelopoulou N, Perrea D, Ampatzidis G, Liapis CD et al. The anti-inflammatory effects of exercise training in patients with type 2 diabetes mellitus.Eur J CardiovascPrevRehabil. 2007;14(6):837-43.
[24]Chen GQ, Mou CY, Yang YQ, Wang S, Zhao ZW. Exercise training has beneficial anti-atrophy effects by inhibiting oxidative stress-induced MuRF1 upregulation in rats with diabetes. Life Sci. 2011; 89(1-2):44-9.
[25]Martín-Cordero L, García JJ, Hinchado MD, Ortega E. The interleukin-6 and noradrenaline mediated inflammation-stress feedback mechanism is dysregulated in metabolic syndrome: effect of exercise. CardiovascDiabetol.2011; 20;10:42.
[26]Howatson G, van Someren KA. The prevention and treatment of exercise-induced muscle damage. Sports Med. 2008;38(6):483-503.
[27]Liao P, Zhou J, Ji LL, Zhang Y. Eccentric contraction induces inflammatory responses in rat skeletal muscle: role of tumor necrosis factor-alpha. Am J Physiol Regul Integr Comp Physiol. 2010;298(3):R599-607.
[28]Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J ApplPhysiol (1985). 2005;98(4):1154-62.
[29]Reynolds TH 4th, Supiano MA, Dengel DR. Resistance training enhances insulin-mediated glucose disposal with minimal effect on the tumor necrosis factor-alpha system in older hypertensives. Metabolism. 2004;53(3):397-402.
[30]Jorge ML, de Oliveira VN, Resende NM, Paraiso LF, Calixto A, Diniz AL et al. The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus.Metabolism. 2011;60(9):1244-52.
[31]Brooks N, Layne JE, Gordon PL, Roubenoff R, Nelson ME, Castaneda-Sceppa C. Strength training improves muscle quality and insulin sensitivity in Hispanic older adults with type 2 diabetes. Int J Med Sci. 2006; 18;4(1):19-27.
[32]Olson TP, Dengel DR, Leon AS, Schmitz KH. Changes in inflammatory biomarkers following one-year of moderate resistance training in overweight women.Int J Obes (Lond). 2007;31(6):996-1003.
[33]Levinger I, Goodman C, Peake J, Garnham A, Hare DL, Jerums G, Selig S. Inflammation, hepatic enzymes and resistance training in individuals with metabolic risk factors.Diabet Med. 2009;26(3):220-7.
[34]Staron, R.S., Hikida R.S, Murray T.F, Nelson MM, Johnson P and Hagerman FC. Assessment of skeletal muscle damage in successive biopsies from strength-trained and untrained men and women.Eur. J. Appl. Physiol. 1992; 65: 258–264.
[35]Roth SM, Martel GF, Ivey FM, Lemmer JT, Metter EJ, Hurley BF, Rogers MA. High-volume, heavy-resistance strength training and muscle damage in young and older women. J Appl Physiol. 2000; 88(3):1112-8.
[36]Zanchi NE, Lira FS, Seelaender M, Lancha-Jr AH. Experimental chronic low-frequency resistance training produces skeletal muscle hypertrophy in the absence of muscle damage and metabolic stress markers. Cell BiochemFunct. 2010; 28(3):232-8.