Cytokine 120 (2019) 99–106

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Myostatin alters with exercise training in diabetic rats; possible interaction with glycosylated hemoglobin and inflammatory cytokines

Fahimeh Kazemi

Department of Exercise Physiology, Faculty of Physical Education and Sport Sciences, Alzahra University, Tehran, Iran

ARTICLE INFO

Keywords:
Aerobic exercise
HbA1c
IL-1β
IL-6
Myostatin
TNF-α
Type 1 diabetes

ABSTRACT

The role of myostatin (MSTN) in the regulation of energy homeostasis has been known and that MSTN inhibition can attenuate the development of diabetes. However, the response of MSTN to exercise in type 1 diabetes (T1DM) is unknown. This study aimed to investigate the alteration of MSTN following aerobic exercise training in diabetic rats and its possible interaction with glycosylated hemoglobin (HbA1c) and inflammatory cytokines. Forty-eight male Wistar rats were divided into non-diabetic untrained, non-diabetic trained, diabetic untrained and diabetic trained groups. To induce T1DM, rats received an intraperitoneal injection of STZ (60 $mg \cdot kg^{-1}$). Treadmill exercise was performed for six weeks, five days/week. HbA1c was estimated, MSTN mRNA expression in skeletal muscle was measured, and plasma MSTN and inflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) were determined. Results revealed a significant decrease of HbA1c and plasma inflammatory cytokines (IL-6, TNF-α and IL-1β) followed by a significant decrease of plasma and skeletal muscle MSTN in diabetic trained rats versus non-diabetic untrained and diabetic untrained rats after the experimental period. Moreover, in diabetic untrained and diabetic trained rats, a significantly positive correlation (change versus change) of plasma MSTN with HbA1c and plasma IL-6, TNF-α and IL-1β was found. In conclusion, this study indicated that aerobic exercise training by a decrease of HbA1c and plasma IL-6, TNF-α and IL-1β could decrease MSTN levels in plasma and skeletal muscle in T1DM. Furthermore, the effective influence of exercise may be reflected by changes of MSTN in diabetic rats.

1. Introduction

Type 1 diabetes (T1DM) is caused by a lack of insulin secretion in the body due to pancreatic β cell damage [1,2]. Exercise training as a therapeutic strategy reduces the progression of T1DM [1], so that altered secretion of myokines including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) may be responsible for the beneficial effects of exercise training [3]. Myokines are muscle-produced secretory proteins acting as hormones either locally in the muscle or an endocrine fashion on other organs such as brain, pancreas, liver or adipose tissue [4]. Myokine named MSTN, or growth/differentiation factor-8 (GDF-8), is a negative regulator of skeletal muscle mass in various animal species and humans [5]. Because MSTN greatly influences skeletal muscle growth and therefore the total volume of metabolically active lean body mass, it can have profound effects on whole body metabolism [6]. Previous studies have shown the increase of MSTN levels in patients with type 2 diabetes [7,8] and in streptozotocin (STZ)-induced T1DM mice [9]. Also, MSTN inhibition in STZ-induced diabetic mice [2] resulted in an improvement in insulin sensitivity. These data suggest the role of MSTN in the regulation of energy homeostasis and that MSTN inhibition can significantly attenuate the development of diabetes.

Evidence indicates the beneficial effects of regular exercise training on MSTN levels in insulin-resistant [10,11] and dysglycaemia [3] in human and animal studies. Hittle et al. 2010 found decreased muscle and plasma MSTN protein levels with aerobic exercise and its relationship with insulin resistance in men [10]. In another study, Bueno et al. 2011 demonstrated the changes in MSTN mRNA expression in obese insulin-resistant rats subjected to exercise [11]. Furthermore, Hjorth et al. 2016 showed reduced MSTN mRNA expression in the skeletal muscle after acute and long-term physical activity, MSTN correlation with physical activity and dysglycaemia and its effect on energy metabolism in human skeletal muscle cells [4]. However, the response of MSTN to exercise in T1DM is not clearly understood. In two recent studies, decreased MSTN mRNA expression [12] and MSTN protein [13] in STZ-induced diabetic rats was found following aerobic exercise training. Notably, the genesis and progression of diabetes occur due to the activation of inflammatory factors, such as inflammatory cytokines [14]. Elevations in markers of inflammatory have been identified in T1DM [1]. Inflammatory cytokine IL-6 is elevated in T1DM

E-mail address: f.kazemi@alzahra.ac.ir.

https://doi.org/10.1016/j.cyto.2019.04.012
Received 4 February 2019; Received in revised form 7 April 2019; Accepted 17 April 2019
Available online 01 May 2019

Table 1
Exercise training protocol on the treadmill.

	Familiarization	week 1	week 2	week 3	week 4	week 5	week 6
Speed (m/min)	10	12	12	16	16	20	20
Duration (min)	10	15	20	25	30	35	40
Grade (%)	0	5	5	5	5	5	5

patients, so that increased this cytokine has an important role in the progression and development of cardiovascular complications [15]. Moreover, TNF-α is a cytokine involved in systemic inflammation and results in acute phase reaction [16]. TNF-α level plays the main role in T1DM [17]. TNF-α is a key pro-inflammatory cytokine with widespread metabolic effects and directly regulate the production of several cardiovascular risk factors [18]. The growing body of evidence shows that interleukine-1 beta (IL-1β) as a pro-inflammatory cytokine plays a key role in the development of diabetes and its cardiovascular complications and IL-1β blockade can be a potential pharmacological approach to treat diabetes [19]. In addition, inflammation negatively affects skeletal muscle health, as observed by the positive correlation of inflammatory factors with muscle wasting [1]. Exercise therapy, rather than drug treatment, has been recommended to combat hyperglycemia [20]. Exercise also elicits anti-inflammatory effects and by decreased inflammation associated with T1DM development [1], so that the reductions in inflammatory cytokines such as IL-6, TNF-α and IL-1β in diabetic rats have been demonstrated following exercise training intervention [14,20,21]. Given the limited studies about MSTN response to exercise in T1DM, the correlation of exercise-induced MSTN with inflammatory cytokines and the importance of IL-6, TNF-α and IL-1β in T1DM, this is the first study to investigate the alteration of MSTN following aerobic exercise training in STZ-induced diabetic rats and its possible interaction with glycosylated hemoglobin (HbA1c) and inflammatory cytokines including IL-6, TNF-α and IL-1β.

2. Materials and methods

Mature male Wistar rats (ten-week-old, 250–280 g) were obtained Pasteur Institute, Tehran, Iran and were kept in a room with a 12-hour light-dark cycle under standardized conditions of temperature (24 ± 2 °C) and humidity (26 ± 2%). Animals had free access to water and pellet rodent diet. The experiment was approved by the Ethical Committee of Shahid Beheshti University of Iran and followed according to the guidelines for the care and use of laboratory animals. Forty-eight animals were divided randomly into four groups (n = 12 per group): non-diabetic untrained, non-diabetic trained, diabetic untrained and diabetic trained.

Experimental diabetes was induced by intraperitoneal (i.p.) injection of 60 $mg \cdot kg^{-1}$ STZ (Sigma, Saint-Louis, MO, USA) dissolved in 0.1 mol/l sodium citrate buffer (pH 4.5) to destroy pancreatic beta cells as a T1DM model [22]. Aspiration was performed before i.p. injection to ensure that the needle tip is at the desired location during this blind procedure. Notably, no material (urine, blood, or digesta) was aspirated the needle. Forty- eight hours after STZ injection [23], the diabetic state was confirmed by elevation of blood glucose to > 250 $mg \cdot dl^{-1}$ as determined by the glucose oxidase method [23]. Blood samples were collected from a cut at the tip of the animal's tail. Moreover, blood glucose levels were measured weekly to ensure that the animals stayed in a hyperglycemic state through all experiments. The rats weighed weekly, and food and water intake were evaluated daily. Diabetic rats showed other symptoms of T1DM such as increased food intake (polyphagia), water intake (polydipsia) and urination (polyuria), and weight loss.

Treadmill exercise was performed for six weeks, five days/week [14]. The animals tolerated the training well and were able to increase running distance and intensity according to the training protocol throughout the study. Initially, non-diabetic trained and diabetic trained rats were familiarized with a treadmill running at low speeds (10 m/min) for 10 min/day for five consecutive days. Training was started at 12 m/min for 15 min on a 5% grade. After that, the speed and duration were increased progressively over the six weeks, until the animals were running 20 m/min for 40 min for last week (Table 1). Non-diabetic untrained and diabetic untrained animals remained sedentary (no exercise on the treadmill) in their cages for the duration of the 6-week training program [14]. Forty-eight hours after the last exercise training session (to minimize the acute effects of the exercise), the rats were anesthetized with pentobarbital sodium (100 mg/kg body weight, i.p.) and plasma samples (at least 5 ml) were taken by an intracardiac puncture from fasting rats. Then blood samples were centrifuged at 3000 rpm for 10 min at 4 °C, and plasma was stored at −80 °C.

Plasma glucose was determined using a rat glucose kit (Pars Azmoon, Tehran, Iran), with intra-assay coefficient variation (InteraCV) of 6.9%, respectively. Plasma insulin levels were analyzed by a rat insulin ELISA kit (Mercodia, Uppsala, Sweden), with InteraCV of 6.4%, respectively. HbA1c was estimated by the method of Nayak and Pattabiraman (1981) [24]. Plasma levels of IL-6, TNF-α and IL-1β were determined by enzyme-linked immunosorbent assay (ELISA), by a DuoSet kit (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. InteraCV of IL-6, TNF-α and IL-1β was 5.7%, 6.1%, 5.9%, respectively.

Plasma MSTN was assessed using a rat MSTN ELISA kit (Biospes, Chongqing, China), with InteraCV of 6,3%, respectively.

MSTN is expressed almost exclusively in skeletal muscle [5]. Moreover, the soleus muscle (type 1; slow-twitch) is mitochondria-rich and mostly utilizes oxidative metabolism for energy generation during aerobic exercises such as swimming and treadmill [25]. For these reasons, in the present study, the soleus muscle was chosen for RNA extraction. Rats were anesthetized with pentobarbital sodium (80 mg/kg body weight, i.p.) and the soleus muscle tissues were collected for RNA extraction using TRIzol. Reverse transcription (RT) was performed on total RNA (500 ng) and the cDNA products were used as a template for quantitative real-time PCR (qRT-PCR) (Rotor-Gene 6000 real-time PCR Detector, Corbett Life Science, Australia). SYBR green was used as a fluorescent label, and primers specificity was tested by applying the melting curve cycle. MSTN mRNA level was normalized to hypoxanthine phosphoribosyltransferase 1 (HPRT1) expression [26]. qRT-PCR conditions were as follows: 95 °C pre-denaturation for 5 min, 95 °C denaturation for 30 s, 60 °C annealing for 30 s, 72 °C elongation for 30 s, 40 cycles, followed by final denaturation at 95 °C for 15 s and final elongation at 60 °C for 1 min HPRT1 was used as an internal control. Reactions were performed in triplicate.

Primer sequences for MSTN	Forward: 5′-TACCACGGAAACAATCATTACCAT-3′
	Reverse: 5′-TGCCATCCGCTTGCATT-3′
Primers sequences for HPRT1	Forward: 5′-GCGAAAGTGGAAAAGCCAAGT-3′
	Reverse: 5′-GCCACATCAACAGGACTCTTGTAG-3′

Before the experimental period, two groups were compared using independent sample t-test. After the experimental period, group comparisons were assessed by two-way analysis of variance (ANOVA) and Tukey post hoc test. Correlation analyses (change versus change) were also performed using the Pearson product-moment correlation coefficient. Significance was accepted at $P \leq 0.05$. All data are reported as means ± SEM. All analyses were done using SPSS V16.0 (SPSS,

Table 2
Polyphagia, polydipsia, skeletal muscle weight and metabolic parameters of rats after the experimental period.

	Non-diabetic untrained	Non-diabetic trained	Diabetic untrained	Diabetic trained
Food intake (g/day)	17.11 ± 4.32	16.82 ± 5.06	32.21 ± 8.09** ††	31.93 ± 6.57** ††
Water intake (ml/day)	34.71 ± 5.4	35.6 ± 4.52	130.14 ± 6.38** ††	128.91 ± 5.86** ††
Dry soleus muscle weight (g)	0.36 ± 0.03	0.38 ± 0.04	0.21 ± 0.02** ††	0.28 ± 0.03* † §
Glucose (mg/dl)	82.05 ± 6.21	77.89 ± 5.32	486.51 ± 17.16** ††	435.85 ± 15.71** †† §§
Insulin (ng/ml)	0.58 ± 0.04	0.65 ± 0.05	0.17 ± 0.02** ††	0.24 ± 0.02** †† §
HbA1c (%)	4.14 ± 0.16	3.85 ± 0.11	11.78 ± 0.3** ††	9.81 ± 0.2** †† §§
IL-6 (pg/ml)	11.49 ± 0.33	10.83 ± 0.3	15.38 ± 0.52** ††	13.11 ± 0.23** †† §
TNF-α (pg/ml)	173.25 ± 9.53	174.33 ± 8.18	197.91 ± 8.72** ††	181.91 ± 7.24* † §
IL-1β (pg/ml)	142.63 ± 6.15	148.79 ± 5.47	201.04 ± 7.61** ††	161.8 ± 6.23** †† §§
MSTN (ng/ml)	44.2 ± 4.08	46.16 ± 3.24	68.41 ± 4.52** ††	51.25 ± 4.69** †† §

Values are means ± SEM (n = 12 rats per group). *P < 0.05,**P < 0.001 versus non-diabetic untrained rats, †P < 0.05,††P < 0.001 versus non-diabetic trained rats, §P < 0.05, §§p < 0.001 versus diabetic untrained rats. HbA1c: glycosylated hemoglobin, IL-6: interleukin-6, IL-1β: interleukin-1 beta, TNF-α: tumor necrosis factor alpha, MSTN: myostatin.

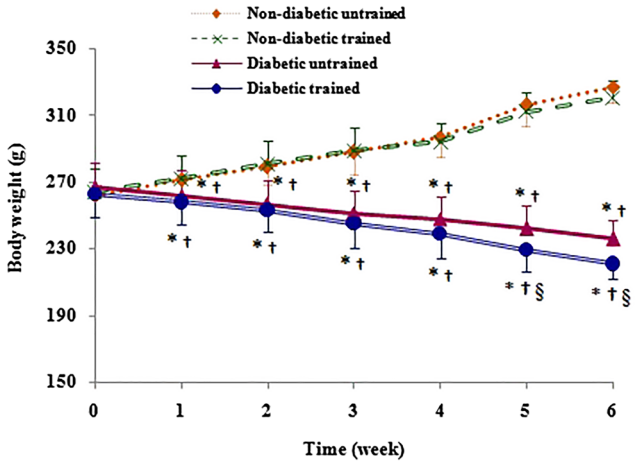

Fig. 1. Body weight during the experimental period. Values are means ± SEM (n = 12 rats per group). *P < 0.001 versus non-diabetic untrained rats, †P < 0.001 versus non-diabetic trained rats, §P < 0.001, versus diabetic untrained rats.

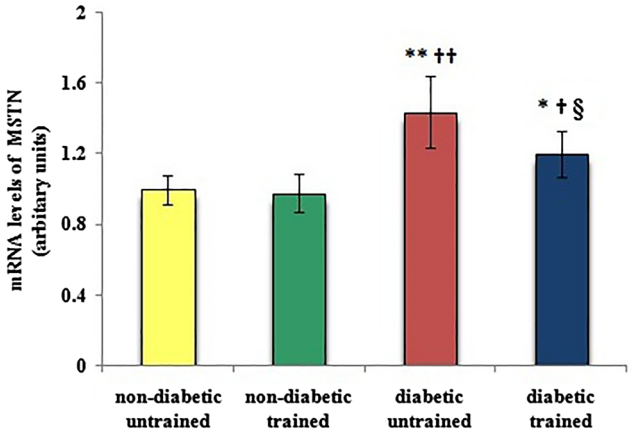

Fig. 2. MSTN mRNA levels in soleus muscle tissue after the experimental period. MSTN mRNA levels were analyzed by real-time PCR in soleus muscle tissue. Results expressed as relative activity in diabetic compared with non-diabetic rats. Each bar represents the means ± SEM. The number of groups: 4 and n = 12 rats per group. *P < 0.05, **P < 0.001 versus non-diabetic untrained rats, †P < 0.05, ††P < 0.001 versus non-diabetic trained rats, §P < 0.05, versus diabetic untrained rats.

Chicago, IL, USA). G*Power software version 3 (Universität Kiel, Germany) was used to calculate sample size and power.

Two-way ANOVA: Power (1-β) = 0.98, α = 0.05, Effect size (f) = 0.7, Number of group = 4, Repetations = 2; Therefore, Total sample size = 48

Pearson correlation: Power (1-β) = 0.92, α = 0.05, Effect size (f) = 1.5; Therefore, Sample size for each group = 12

3. Results

In STZ-induced diabetic rats, polydipsia and polyuria increased significantly compared with non-diabetic rats. Food intake: 16.49 ± 2.14 g/day versus 31.53 ± 2.3 g/day, P = 0.027; Water intake: 128.23 ± 9.15 ml versus 34.84 ± 5.31 ml, P = 0.036; Urine volume: 115.38 ± 7.2 ml versus 14.61 ± 2.12 ml, P = 0.018. In all experimental period, the food and water intake in diabetic untrained and diabetic trained rats were significantly higher than that of non-diabetic rats. Exercise training had no significant effect on mean daily food and water intake in diabetic untrained and diabetic trained rats (Table 2).

There was no significant difference in body weight between non-diabetic untrained and non-diabetic trained rats. Body weight of STZ-induced diabetic rats was significantly decreased after the injection. Exercise training caused a significant reduction in body weight of diabetic rats (from week 5 to week 6). Body weight reduction in diabetic trained rats was significantly higher than in diabetic untrained rats. Body weight of diabetic rats (trained and untrained) was significantly lower than non-diabetic rats (Fig. 1). Dry soleus muscle weight of non-diabetic rats was similar. This variable in diabetic trained and diabetic untrained rats was significantly lower than those in non-diabetic rats. Moreover, dry soleus muscle weight in diabetic trained rats was significantly higher than those in diabetic untrained rats (Table 2).

Exercise training did not change plasma levels of glucose and insulin and HbA1c in non-diabetic rats. Exercise training significantly improved glycemic profile in diabetic rats. Plasma glucose and HbA1c significantly decreased, and plasma insulin significantly increased in diabetic trained rats versus diabetic untrained rats. A significant increase in plasma glucose and HbA1c and a significant decrease in plasma insulin were found in diabetic untrained and diabetic trained rats versus non-diabetic rats (Table 2).

In non-diabetic rats, plasma levels of IL-6, TNF-α and IL-1β were similar, but a significant increase of plasma IL-6, TNF-α and IL-1β was found in diabetic untrained and diabetic trained rats versus non-diabetic rats. Moreover, plasma IL-6, TNF-α and IL-1β significantly decreased in diabetic trained rats versus diabetic untrained rats (Table 2).

Exercise training did not influence plasma MSTN in non-diabetic rats. Plasma MSTN in diabetic rats (untrained and trained) was significantly higher than that found in non-diabetic rats. In diabetic trained rats, plasma MSTN significantly decreased versus diabetic untrained rats (Table 2).

In both groups of diabetic rats, MSTN mRNA levels in soleus muscle tissue were significantly higher than non-diabetic rats. Diabetic trained

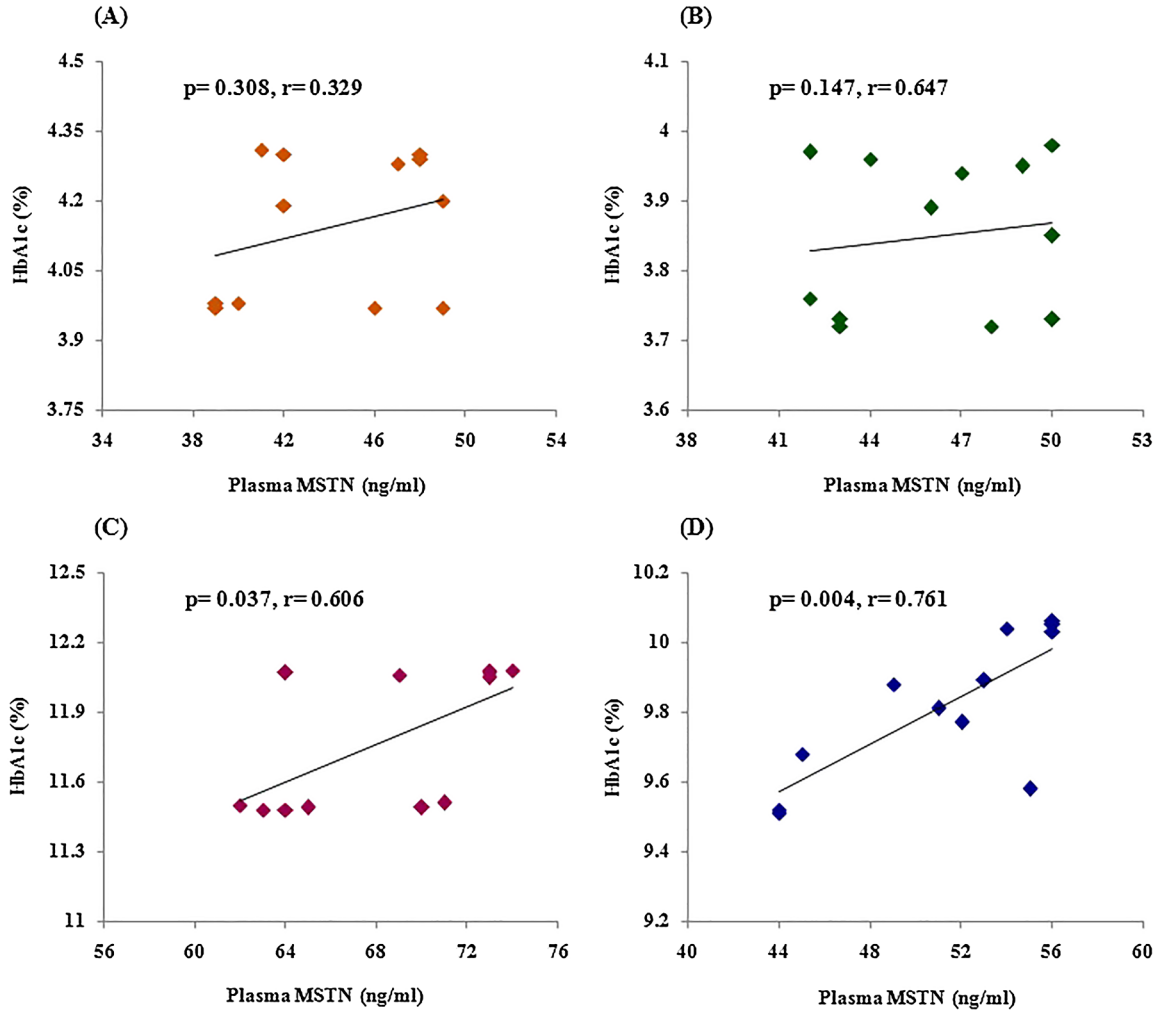

Fig. 3. Correlation (change versus change) between plasma MSTN and HbA1c in non-diabetic untrained (A), non-diabetic trained (B), diabetic untrained (C) and diabetic trained rats (D) after the experimental period (n = 12 per group).

rats showed significantly lower MSTN mRNA levels in soleus muscle tissue than diabetic untrained rats; in contrast, this variable did not differ between non-diabetic untrained and non-diabetic trained rats (Fig. 2).

No significant correlation of plasma MSTN with HbA1c (Fig. 3A, B) and plasma levels of IL-6 (Fig. 4A, B), TNF-α (Fig. 5A, B) and IL-1β (Fig. 6A, B) was found in non-diabetic untrained and non-diabetic trained rats. In diabetic untrained and diabetic trained rats, there was a significantly positive correlation between plasma MSTN and HbA1c (Fig. 3C, D). Furthermore, a significantly positive correlation of plasma MSTN with plasma levels of IL-6 (Fig. 4C, D), TNF-α (Fig. 5C, D) and IL-1β (Fig. 6C, D) was found in diabetic untrained and diabetic trained rats.

4. Discussion

Our results showed that the levels of MSTN in plasma and skeletal muscle of STZ-induced diabetic rats increased compared with non-diabetic rats. This finding is in agreement of Chen et al. 2009 who reported increased MSTN mRNA expression in skeletal muscle of STZ-induced diabetic mice, suggesting that up-regulation of MSTN expression contributes to muscle atrophy in insulin deficiency [9]. In the present study, increased plasma MSTN in diabetic rats may be due to increased MSTN expression in skeletal muscle, so that MSTN predominantly express by skeletal muscle and skeletal muscle MSTN can contribute to circulating MSTN.

The current study also demonstrated that aerobic exercise training decreased the levels of MSTN in plasma and skeletal muscle of diabetic rats compared with non-diabetic rats. There is limited evidence that MSTN levels are regulated by exercise training in T1DM [12,13]. A recent study indicated that 4-week swimming exercise (45 min at 9:00 and 17:00 h, five days/week) increased MSTN mRNA expression in the muscle of STZ-induced diabetic compared with non-diabetic untrained rats [12]. This result suggests that exercise training can modulate the increase MSTN expression in muscle skeletal of diabetic rats and indicated that MSTN might be involved in energy homeostasis. We also found a significantly positive correlation (change versus change) between plasma MSTN levels and HbA1c in diabetic untrained and diabetic trained rats after the experimental period, whereas there was no significant correlation between plasma MSTN levels with HbA1c in non-diabetic untrained and non-diabetic trained rats. Reduced plasma MSTN followed by decreased HbA1c in diabetic trained rats is demonstrating that MSTN has been implicated in metabolic adaptation to physiological stimuli, such as exercise training, which is linked to improved glucose homeostasis [13]. Altered MSTN expression in the skeletal muscle may be responsible for improved metabolic regulation and HbA1c after exercise training. The specific mechanism responsible for the reduction of exercise-induced MSTN in T1DM is not known; however, MSTN and aerobic exercise have similar mechanisms, so that both stimulate adenosine monophosphate-activated protein kinase

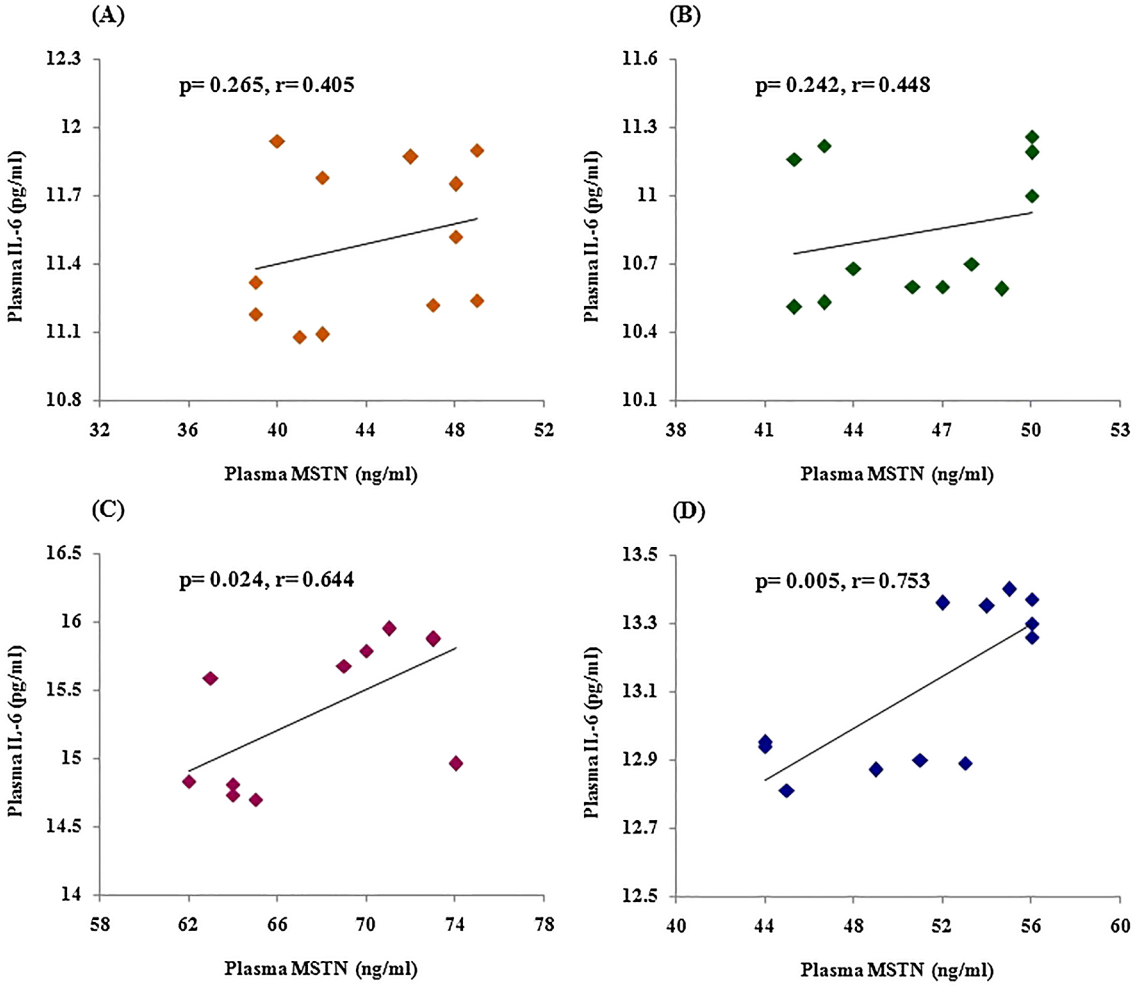

Fig. 4. Correlation (change versus change) between plasma levels of MSTN and IL-6 in non-diabetic untrained (A), non-diabetic trained (B), diabetic untrained (C) and diabetic trained rats (D) after the experimental period (n = 12 per group).

(AMPK) activity leading to enhance glucose uptake and improve insulin sesitivity [13]. In other words, MSTN may influence insulin sensitivity through direct effects on glucose uptake independent of its effects on muscle growth. This raises the possibility that the well-reported changes in MSTN expression in response to exercise training may modulate or in some way contribute to the beneficial effects of exercise via direct effects on skeletal muscle metabolic function as well as via effects on other tissues [6].

We also found that diabetes induced a decrease of skeletal muscle weight in diabetic untrained rats compared with non-diabetic rats. However, exercise training increased skeletal muscle weight in diabetic rats in comparison to diabetic untrained rats. MSTN is a negative regulator of skeletal muscle growth [4] such that diabetic trained rats in which the skeletal muscle MSTN expression is declined display significant increases in muscle mass. This finding is inconsistent with previous data, indicating that by decreasing the MSTN expression in skeletal muscle of STZ-induced mice, the loss of skeletal muscle and whole body mass was decreased [2]. Notable, at least part of MSTN effects on metabolism can be associated with its influence over skeletal muscle growth and thus on the total amount of metabolically active lean body tissue [5]. In addition to inhibiting muscle hypertrophy, MSTN regulates metabolism [6]. MSTN is attributable to insulin sensitivity, and its inhibition can significantly attenuate the progression of diabetes [4,6]. This may be due to an increased muscle mass or a more direct effect of MSTN on energy metabolism [4]. Several studies have indicated negative correlations between MSTN levels in skeletal muscle and insulin sensitivity in humans [26,27]. Moreover, MSTN inhibition in STZ-induced diabetic mice improved insulin sensitivity [2]. Therefore, the maintenance of muscle mass by exercise training, along with the changes in glucose handling abilities, in diabetic rats may result in an improvement in HbA1c.

In the current study, plasma levels of cytokines IL-6, TNF-α and IL-1β were increased in diabetic rats compared with non-diabetic rats. This finding is in agreement with previous studies [14,20,21], indicating that inflammatory cytokines IL-6, TNF-α and IL-1β increase in diabetic rats and that the inflammation plays a critical role in the genesis and progression of diabetes. Increased levels of inflammatory mediators such as pro-inflammatory cytokines have been indicated in diabetes to be a consequence and cause of hyperglycemia [14,20,21]. Hyperglycemia in diabetes results in the production of free radicals, induction of inflammation and reduction of different defense mechanisms [14]. The results of the present study also showed a significant decrease in plasma IL-6, TNF-α and IL-1β in diabetic trained rats compared with diabetic untrained rats. Inconsistent with our finding, recent studies suggested that regular exercise training has marked anti-inflammatory effects on T1DM [14,20,21]. The results regarding the anti-inflammatory effects of exercise are different, suggesting differences in the type of exercise, duration of exercise, the intensity of exercise, endurance capacity and muscle morphology [14]. Regular exercise is physiologically critical in reducing diabetes-induced inflammation. The beneficial effects of exercise training are mediated by control of plasma levels of cytokines such as IL-6, TNF-α and IL-1β

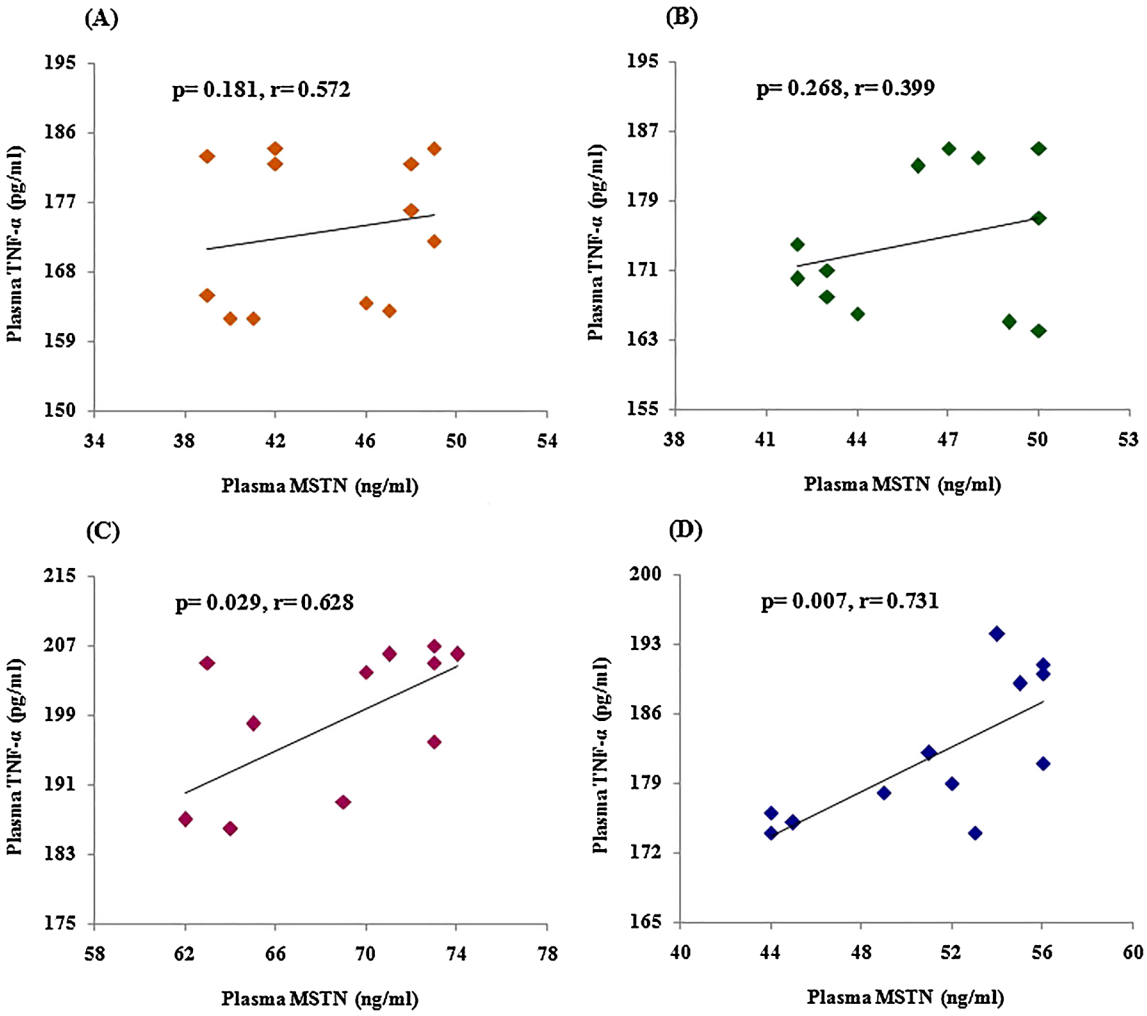

Fig. 5. Correlation (change versus change) between plasma levels of MSTN and TNF-α in non-diabetic untrained (A), non-diabetic trained (B), diabetic untrained (C) and diabetic trained rats (D) after the experimental period (n = 12 per group).

[14,20,21]. Therefore, the prescription of exercise by decreased plasma levels of inflammatory mediators may be an important therapeutic strategy to control the pathogenesis of T1DM. Furthermore, we found no significant correlation (change versus change) of plasma MSTN with plasma levels of IL-6, TNF-α and IL-1β in non-diabetic untrained and non-diabetic trained rats after the experimental period, whereas there was a significantly positive correlation between plasma MSTN with plasma levels of IL-6, TNF-α and IL-1β in diabetic untrained and diabetic trained rats. This study is the first suggesting that exercise training-induced MSTN correlated positively with plasma IL-6, TNF-α and IL-1β in T1DM, reflecting that a decrease in plasma IL-6, TNF-α and IL-1β following exercise training may reduce plasma MSTN. Inflammation negatively affects skeletal muscle health, as observed by the positive correlation of inflammatory factors with muscle wasting [1]. In T1DM, muscle wasting essentially results from insulin deficiency; there are multiple triggers to muscle wasting including inflammatory cytokines, which ultimately activate protein catabolic pathways [28]. IL-6 as a myokine may play a significant role in MSTN-mediated glucose metabolism [29]. MSTN may also affect glucose uptake indirectly through its effects on TNF-α expression, which can antagonize the effects of insulin on glucose uptake [30]. The evidence is demonstrating that inflammation stimulates muscle wasting and that the infusion of IL-6, TNF-α and IL-1β into rodents induces muscle wasting, while neutralization of cytokines by genetic or pharmacological approaches results in attenuating muscle wasting [19]. In T1DM, aerobic and resistance exercise training can combat muscle wasting by improvement of blood flow through muscle, improvement of insulin responsiveness and increase of muscle mass [31]. These data show that in parallel with the decrease of plasma IL-6, TNF-α and IL-1β, MSTN may result in improvement of HbA1c in T1DM. Collectively, these data build the foundation for considering the inhibition of exercise training-induced myokine by exercise to decrease inflammatory cytokines and improve HbA1c in T1DM.

There is a limitation to the present study. MSTN, IL-6, TNF-α and IL-1β levels were determined at one-time point (48 hours after the exercise training).

In conclusion, the present study indicated that exercise training effects on MSTN in T1DM and exercise-induced MSTN correlates with HbA1c and inflammatory cytokines. It appears that aerobic exercise training by the decrease of HbA1c and plasma levels of IL-6, TNF-α and IL-1β can reduce plasma MSTN and skeletal muscle MSTN expression in diabetic rats. Therefore, exercise-induced MSTN may be an efficient tool for the prevention and treatment of T1DM. Indeed, this study suggests that MSTN inhibition through exercise could be as a part of any therapeutic strategy in T1DM as a means to improve HbA1c and reduce inflammatory cytokines.

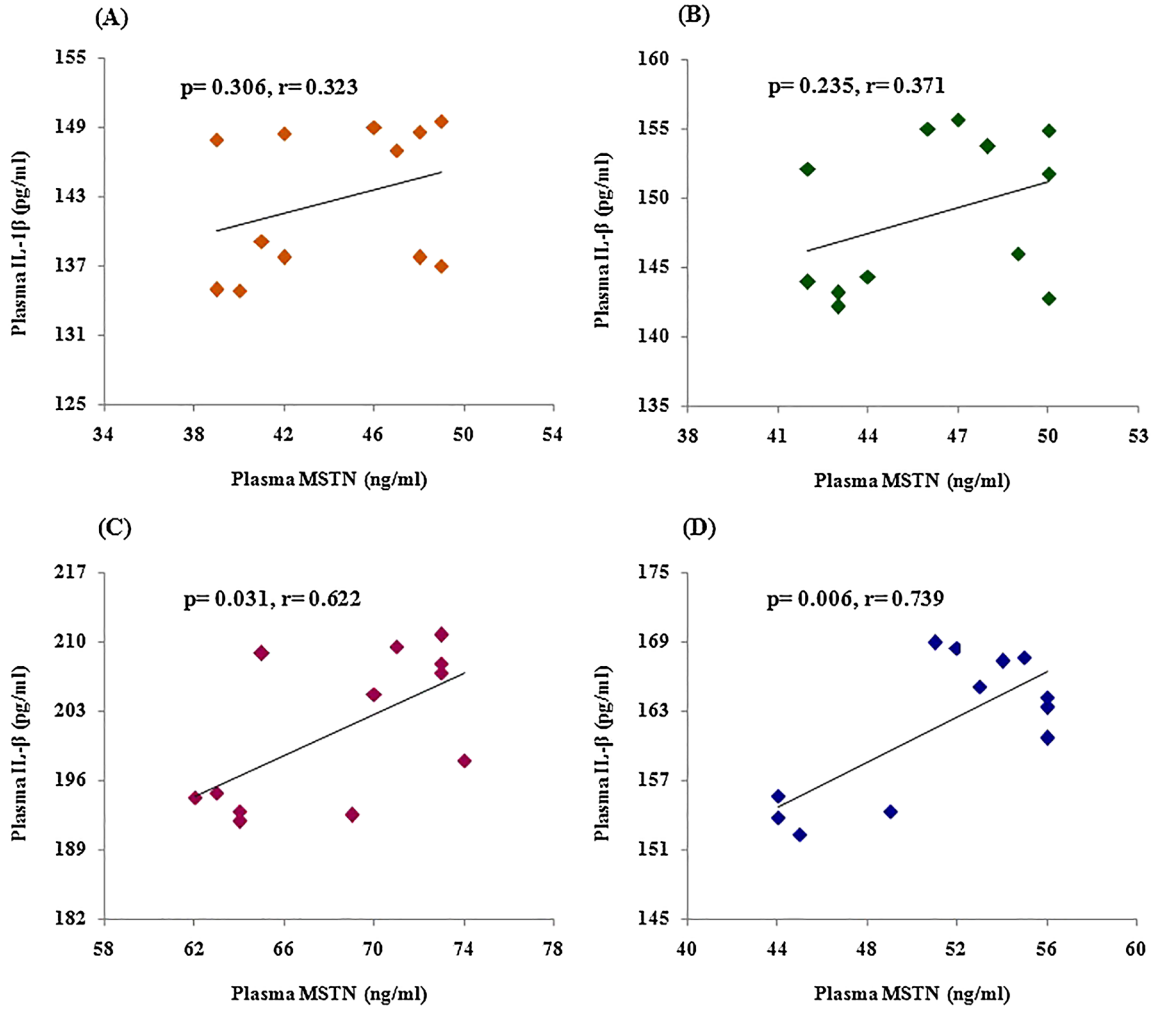

Fig. 6. Correlation (change versus change) between plasma levels of MSTN and IL-1β in non-diabetic untrained (A), non-diabetic trained (B), diabetic untrained (C) and diabetic trained rats (D) after the experimental period (n = 12 per group).

Acknowledgements

The author declares that she has no conflict of interest.

References

[1] S.K. Coleman, I.A. Rebalka, D.M. D'Souza, et al., Skeletal muscle as a therapeutic target for delaying type 1 diabetic complications, World J Diabetes 6 (17) (2015) 1323–1336.
[2] S.K. Coleman, I.A. Rebalka, D.M. D'Souza, et al., Myostatin inhibition therapy for insulin-deficient type 1 diabetes, Sci Rep 6 (2016) 32495.
[3] B.K. Pedersen, The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control, Essays Biochem 42 (2006) 105–117.
[4] M. Hjorth, S. Pourteymour, S.W. Görgens, et al., Myostatin in relation to physical activity and dysglycaemia and its effect on energy metabolism in human skeletal muscle cells, Acta Physiol (Oxf) 217 (1) (2016) 45–60.
[5] A.C. McPherron, A.M. Lawler, S.J. Lee, Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member, Nature 387 (6628) (1997) 83–90.
[6] D.L. Allen, D.S. Hittel, A.C. Mcpherron, Expression and function of myostatin in obesity, diabetes, and exercise adaptation, Med Sci Sports Exerc 43 (10) (2011) 1828–1835.
[7] C. Brandt, A.R. Nielsen, C.P. Fischer, et al., Plasma and muscle myostatin in relation to type 2 diabetes, PLoS One 7 (5) (2012).
[8] F. Wang, Y. Liao, X. Li, et al., Increased circulating myostatin in patients with type 2 diabetes mellitus, J Huazhong Univ Sci Technolog Med Sci 32 (4) (2012) 534–539.
[9] Y. Chen, L. Cao, J. Ye, et al., Upregulation of myostatin gene expression in streptozotocin-induced type 1 diabetes mice is attenuated by insulin, Biochem Biophys Res Commun 388 (1) (2009) 112–116.
[10] D.S. Hittel, M. Axelson, N. Sarna, et al., Myostatin decreases with aerobic exercise and associates with insulin resistance, Med Sci Sports Exerc 42 (11) (2010) 2023–2029.
[11] P.G. Bueno, D. Bassi, D.G. Contrera, et al., Post-exercise changes in myostatin and actRIIB expression in obese insulin-resistant rats, Mol Cell Endocrinol 339 (1–2) (2011) 159–164.
[12] D.B. Dutra, P.G. Bueno, R.N. Silva, et al., Expression of myostatin, myostatin receptors and follistatin in diabetic rats submitted to exercise, Clin Exp Pharmacol Physiol 39 (5) (2012) 417–422.
[13] D. Bassi, G. Bueno Pde, K.O. Nonaka, et al., Exercise alters myostatin protein expression in sedentary and exercised streptozotocin-diabetic rats, Arch Endocrinol Metab 59 (2) (2015) 148–153.
[14] J.S. Kim, Y.H. Lee, J.C. Kim, et al., Effect of exercise training of different intensities on anti-inflammatory reaction in streptozotocin-induced diabetic rats, Biol Sport 31 (1) (2014) 73–79.
[15] G. Targher, L. Zenari, L. Bertolini, et al., Elevated levels of interleukin-6 in young adults with type 1 diabetes without clinical evidence of microvascular and macrovascular complications, Diabetes Care 24 (5) (2001) 956–957.
[16] L.C. Gahring, N.G. Carlson, R.A. Kulmar, et al., Neuronal expression of tumor necrosis factor alpha in the murine brain, Neuroimmunomodulation 3 (5) (1996) 289–303.
[17] Y.C. Qiao, Y.L. Chen, Y.H. Pan, et al., The change of serum tumor necrosis factor alpha in patients with type 1 diabetes mellitus: a systematic review and meta-analysis, PLoS One 12 (4) (2017).
[18] V. Mohamed-Ali, J.H. Pinkney, S.W. Coppack, Adipose tissue as an endocrine and paracrine organ, Int J Obes Relat Metab Disord 22 (12) (1998) 1145–1158.
[19] C. Peiró, Ó. Lorenzo, R. Carraro, et al., IL-1β inhibition in cardiovascular complications associated to diabetes mellitus, Front Pharmacol 8 (2017) 363.
[20] M.F. Belotto, J. Magdalon, H.G. Rodrigues, et al., Moderate exercise improves leucocyte function and decreases inflammation in diabetes, Clin Exp Immunol 162 (2) (2010) 237–243.
[21] J.R. Bortolon, A.J. Silva Junior, G.M. Murata, et al., Persistence of inflammatory response to intense exercise in diabetic rats, Exp Diabetes Re 2012 (2012).
[22] Z. Lappalainen, J. Lappalainen, N.K. Oksala, et al., Diabetes impairs exercise training-

associated thioredoxin response and glutathione status in rat brain, 1985, J Appl Physiol 106 (2) (2009) 461–467.
[23] M.M. Allam, M.H. Muhammad, Effects of moderate exercise training and detraining on diabetic peripheral neuropathy in streptozotocin-induced diabetic rats, Benha Med J 35 (2018) 50–156.
[24] Y.X. Wang, C.L. Zhang, R.T. Yu, et al., Regulation of muscle fiber type and running endurance by PPARdelta, PLoS Biol 2 (10) (2004).
[25] S.S. Nayak, T.N. Pattabiraman, A new colorimetric method for the estimation of glycosylated hemoglobin, Clin Chim Acta 109 (1981) 267–274.
[26] A.S. Ryan, G. Li, J.B. Blumenthal, et al., Aerobic exercise + weight loss decreases skeletal muscle myostatin expression and improves insulin sensitivity in older adults, Obesity (Silver Spring) 21 (7) (2013) 1350–1356.
[27] D.S. Hittel, J.R. Berggren, J. Shearer, et al., Increased secretion and expression of myostatin in skeletal muscle from extremely obese women, Diabetes 58 (1) (2009) 30–38.
[28] B. Workeneh, M. Bajaj, The regulation of muscle protein turnover in diabetes, Int J Biochem Cell Biol 45 (10) (2013) 2239–2244.
[29] F. Kazemi, The correlation of resistance exercise-induced myostatin with insulin resistance and plasma cytokines in healthy young men, J Endocrinol Invest 39 (4) (2016) 383–388.
[30] D.E. Moller, Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes, Trends Endocrinol Metab 11 (6) (2000) 212–217.
[31] M.S. Bssil, R. Gougeon, Muscle protein anabolism in type 2 diabetes, Curr Opin Clin Nutr Metab Care 16 (1) (2013) 83–88.