The effect of moderate endurance training on gastrocnemius retinol-binding protein 4 and insulin resistance in streptozotocin-induced diabetic rats

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(Received: July 5, 2017; Revised manuscript received: August 1, 2017; Accepted: August 22, 2017)

Abstract: *Background:* Retinol-binding protein 4 (RBP4) is suggested to be involved in the occurrence of insulin resistance. There are contradictory studies about the effects of exercise training on RBP4 levels and insulin resistance. Hence, we designed this study to investigate the impact of moderate endurance training on gastrocnemius RBP4 and insulin resistance in streptozotocin (STZ)-induced diabetic rats. *Method:* Forty male albino Wistar rats were randomly divided into four groups: healthy control (HC), diabetic control (DC), healthy training (HT), and diabetic training (DT). Animals in HT and DT groups ran on a treadmill on the basis of overload principle for 6 weeks, three sessions per week. Rats in DC and DT groups are affected by diabetes using STZ (50 mg/kg of body weight). Gastrocnemius RBP4 content was measured using an enzyme-linked immunosorbent assay kit. Data were analyzed by one-way analysis of variance at $P < 0.05$ level. *Results:* Serum blood glucose level ($P = 0.001$) and insulin resistance ($P = 0.001$) increased in DC compared with HC group, whereas serum insulin ($P = 0.001$) and gastrocnemius RBP4 ($P = 0.001$) reduced. However, there were no significant differences between serum blood glucose level ($P = 0.384$), insulin resistance ($P = 0.999$), and RBP4 ($P = 0.999$) content in DT compared with HT group. *Conclusion:* Moderate endurance training reduces blood glucose level and subsequently improves insulin sensitivity by decreasing gastrocnemius RBP4 content independent of insulin.

Keywords: endurance training, retinol-binding protein 4, insulin resistance, diabetic, rat

Introduction

There is now a substantial body of evidence to suggest that destruction of beta cell, decreased levels of insulin and its receptors, and decreased content of glucose transporters (GLUTs) resulted in insulin resistance [1–3]. Current evidence has reported that retinol-binding protein 4 (RBP4) is also one of the mechanisms involved in the incidents of diabetes [2, 4–6].

RBP4 is a newly discovered fat-derived adipokine [2] that belongs to the lipocalin family [5] and is encoded by the RBP4 gene [6]. RBP4 specifically binds to retinol [7] and transthyretin [4] in blood stream [8]. Its single known function is to deliver vitamin A to tissues via the blood [9]. Recent convincing data in animals and humans suggest a strong causal link between RBP4 and insulin resistance [2], type 2 diabetes [10, 11], and metabolic syndrome [11]. In this context, it has been revealed that the injection of recombinant RBP4 or transgenic overexpression of RBP4 in healthy mice decreases insulin sensitivity [12]. However, RBP4 levels can be normalized by insulin sensitizers and fenretinide, which reverse insulin resistance in obese rodents [12]. In rodents, it has been demonstrated that elevated serum levels of RBP4 were associated with insulin resistance in both liver and skeletal muscle [12]. In humans, subjects with normal glucose tolerance had lower levels of circulating RBP4

than people with impaired glucose tolerance and type 2 diabetes [9, 11–13]. In fact, RBP4 increases gluconeogenesis in liver of mouse and impairs insulin signaling in muscle [8].

Different approaches of weight loss [14, 15], insulin sensitizers [12], and exercise training [10, 16, 17] have been used to reduce RBP4 levels and subsequently improve insulin sensitivity. In this regard, studies have been reported that resistance training with moderate [16, 17] and high intensity [10] reduce RBP4 levels and improve insulin sensitivity in healthy [10] and diabetic [16, 17] postmenopausal females. In addition, improvement in insulin sensitivity following moderate- to high-intensity aerobic exercise [5, 9] attributed to reduction in RBP4 level in obese males [9] and females [5]. Moreover, running on treadmill resulted in a reduction in protein and gene expression of RBP4 in serum [18, 19] and visceral and subcutaneous fat tissue in diabetic [2] and spontaneously hypertensive [18, 19] rats. In contrast, in other studies, it has been reported that neither moderate to intensive aerobic exercise [4, 6, 17] nor walking exercise [16] had any significant effect on fasting glucose, insulin, resistance insulin, and circulating levels of RBP4 in females with type 2 diabetes [16, 17] and healthy athletes [4, 6].

Research indicated that high level of RBP4 resulted in insulin resistance [2, 10]. Therefore, to improve insulin sensitivity, it is important to monitor one's RBP4 levels through an appropriate exercise training program. However, the effects of exercise training on RBP4 levels have remained controversial. In addition, current studies suggested that RBP4, as a myokine, was released by skeletal muscle [2]. However, changes of RBP4 in skeletal muscle following exercise training and its possible effects on insulin resistance have not yet been well examined. Hence, this study set out to investigate the effect of moderate endurance training on gastrocnemius RBP4 and insulin resistance in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Animals and diabetic procedure

The present experimental protocol was approved by the Ethics Committee of Ilam University of Medical Sciences in Iran. Forty male albino Wistar rats (270–340 g) were purchased from Ilam University of Medical Sciences in Iran. Rats were kept in animal house with standard condition (12:12 h dark/light cycle) at 21 ± 2 °C for 2 weeks. Then, all rats taught to run on motor-driven treadmill (5 days at 10 min a day at a speed of 10 m/min) [1]. After the period of acclimatization, 20 rats were randomly affected by diabetes using streptozotocin (STZ; ALX-380-010-G001, Enzo Life Sciences, Farmingdale, NY, USA) at a dosage of 50 mg/kg body weight. To ensure the occurrence of insulin resistance, glucose tolerance test was performed by a glucometer (Easy Glucometer, South Korea) on animals after a 12-h fasting. Results showed the presence of insulin resistance (glucose greater than 300 mg/dl) [1] in all animals. Finally, diabetic rats were randomly divided into equal groups ($n = 10$) of diabetic control (DC) and diabetic training (DT). Also, healthy rats were randomly divided into equal groups ($n = 10$) of healthy control (HC) and healthy training (HT).

Exercise training protocol

Endurance exercise training performed on motor-driven treadmill (Technic-Azma Co., Tabriz, Iran) on the basis of overload principle for 8 weeks, three sessions per week [1]. Rats in DT and HT groups ran at a speed of 12 m/min for 20 min on the first week, and the duration increased with 5 min/week until 50 min was achieved by the eighth week. In addition, running speed increased at 1 m/min/week until 18 m/min was achieved by the eighth week. Throughout the training period, the slope of the treadmill was considered 0°. At the beginning and end of endurance exercise training, warm-up and cool-down were performed for 3 min at 7 m/min [1].

Sample collection and biochemical estimations

Fasting rats were euthanized under anesthesia induced by ether inhalation 48 h after the last exercise training session. An amount of 5 ml of blood was taken directly through cardiac puncture. Blood samples were immediately centrifuged (Eppendorf Centrifuge, Germany) at 10,000 × *g* for 10 min at 2–8 °C. The gastrocnemius muscle of each rat was removed and washed by normal saline and finally homogenized (Heidolph Homogenizer, Germany). Samples were rapidly submerged in liquid nitrogen tanks (MVE XC 34/18 Model, USA) for 2 min and finally kept at −80 °C (GFL Upright Freezer, Germany) until the time of experimentation.

The serum insulin level (Mercodia Co., Sweden) and gastrocnemius RBP4 content (Cusabio Biotech Co., Ltd. Sino-American) were determined using commercial enzyme-linked immunosorbent assay kits. All analyses were performed in accordance with the manufacturers' recommendations. The sensitivities of the kits were 0.015 µg/L and 15.6 ng/ml for insulin and RBP4, respectively. Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated according to the following equation: [glucose (mg/dl) × insulin (µU/ml)/405].

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (software, version 16.0, SPSS Inc., Chicago, IL, USA) and expressed as means ± standard deviation (SD). After the confirmation of normality by Shapiro–Wilk's test, statistical significance was determined by one-way analysis of variance at $P < 0.05$. Furthermore, Tukey's *post-hoc* comparison was used to test the differences between groups.

Results

The results of this study revealed significant difference in body weight ($F = 7.68$, $P = 0.001$), glucose ($F = 69.55$, $P = 0.001$), insulin ($F = 15.29$, $P = 0.001$), insulin resistance ($F = 10.92$, $P = 0.001$), and RBP4 ($F = 9.67$, $P = 0.001$) in the end of exercise training protocol.

The results indicated that body weight decreased significantly in DC (283 ± 15 g) group more than HC group (308 ± 17 g) ($P = 0.011$). However, there was no significant difference between body weight rats in HT (317 ± 7 g) and DT (305 ± 15 g) groups ($P = 0.714$) *(Fig. 1)*.

Serum glucose level increased significantly in DC (370 ± 84 mg/dl) group than in HC (83 ± 10 mg/dl) group ($P = 0.001$). However, there was no significant difference between serum glucose level in HT (86 ± 8 mg/dl) and DT (123 ± 20 mg/dl) groups ($P = 0.384$) *(Fig. 2A)*. Serum insulin level of DC (0.362 ± 0.1 µU/ml) group was significantly lower than HC (0.630 ± 0.06 µU/ml) group ($P = 0.001$). In addition, serum insulin level of DT (0.380 ± 0.01 µU/ml) group was significantly lower than HT (0.569 ± 0.1 µU/ml) group ($P = 0.001$) *(Fig. 2B)*. Insulin resistance in DC (0.432 ± 0.2) group was higher compared with HC (0.136 ± 0.01) group ($P = 0.001$). However, the results showed that there was no significant difference between insulin resistance in DT (0.156 ± 0.04) and HT (0.154 ± 0.02) ($P = 0.999$) groups *(Fig. 2C)*.

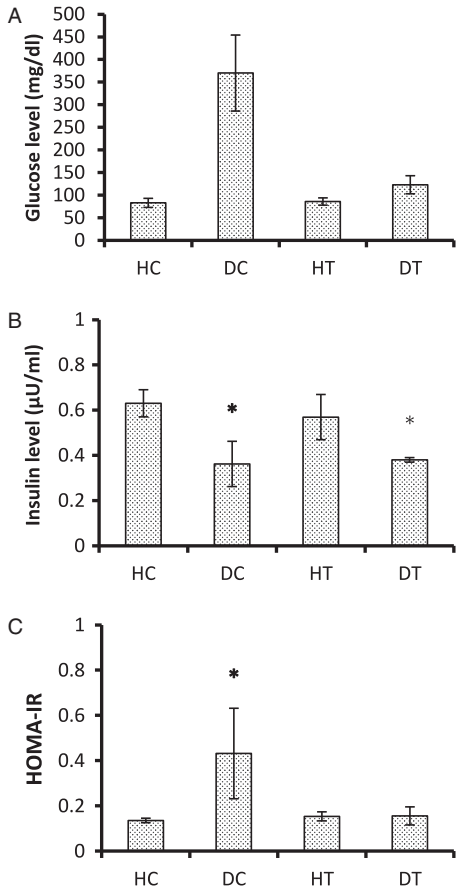

Fig. 2. Changes in serum levels of glucose (A), insulin (B), and HOMA-IR (C) in healthy and diabetic rats following moderate endurance training. Abbreviations are the same as are denoted in the legend of *Fig. 1*. * Significant difference from HC at $P < 0.05$

Finally, gastrocnemius content of RBP4 in DC group (879 ± 107 pg/mg) was significantly higher compared with HC (493 ± 204 pg/mg) group ($P = 0.001$). In contrast, there was no significant difference between RBP4 content in DT (500 ± 136 pg/mg) and HT (503 ± 180 pg/mg) groups ($P = 0.999$) *(Fig. 3)*.

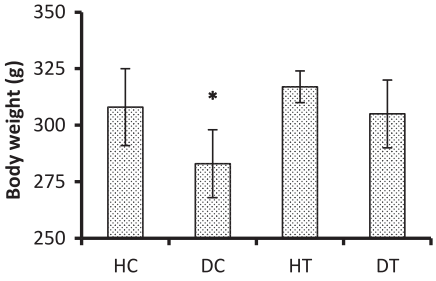

Fig. 1. Animals' body weight in healthy and diabetic rats following moderate endurance training. HC: healthy control; DC: diabetic control; HT: healthy training; DT: diabetic training. * Significant difference from HC at $P < 0.05$

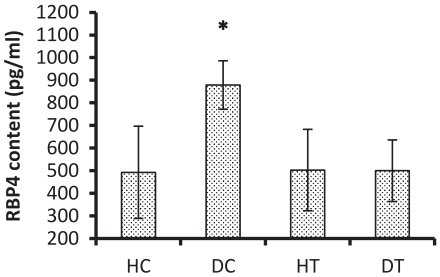

Fig. 3. RBP4 changes in gastrocnemius muscle of healthy and diabetic rats following moderate endurance training. Abbreviations are the same as denoted in the legend of *Fig. 1*. * Significant difference from HC at $P < 0.05$

Discussion

Previous studies have reported various mechanisms involved in diabetes improvement following different types of exercise training [1, 3, 20]. Here, in an experimental animal model, it was revealed another mechanism is involved in improvement of diabetes following exercise training. In reality, the results of this study showed that moderate endurance training through reduction in RBP4 content of gastrocnemius muscle improves insulin resistance in STZ-induced diabetic rats.

Although diabetes causes weight loss in STZ-induced diabetic rats, endurance exercise training prevented weight loss caused by diabetes. Our findings are consistent with other studies that reported an increase in body weight following low- and high-intensity aerobic [21] and resistance [3] exercise training in diabetic rats. In addition, Holten et al. [20] showed an increase in body weight in diabetic human subject following progressive resistance exercise training. Neuropathy is one of the most common consequences of diabetes. In fact, neuropathy induced by diabetes mellitus reduces recruitment and firing motor unit, which subsequently reduces strength and muscle mass [3, 20]. Other mechanism associated with weight loss in diabetic patients is an increase in inflammatory cytokines [3, 21]. Studies have suggested that interleukin 6 and C-reactive protein resulted in diabetic neuropathy [3, 21]. In contrast, muscle contractures or exercise training enhance fire and recruitment of motor unit by reducing and increasing inflammatory and anti-inflammatory cytokines, respectively [3, 21].

The results of this study showed a reduction in insulin level in STZ-diabetic rats, which subsequently increased serum glucose level and insulin resistance. However, endurance training reduced serum glucose level and improved insulin sensitive. In line with our results, it has been reported that a reduction in glucose level and insulin resistance following resistance [20], aerobic [22], and combined [23] exercise training in diabetic patients. Decrease in serum glucose level and insulin resistance depends on glucose uptake by insulin and non-insulin-dependent tissues [1]. However, exercise training did not change insulin levels in STZ-diabetic rats. Thus, the observed change in serum glucose level induced by exercise training is explained in the light of the other mechanisms. In this context, it has been demonstrated that muscle contractures or exercise training increase expression and translocation of GLUT4 from intracellular stores to the cell membrane [1]. Reduction in gastrocnemius RBP4 induced by exercise training appears to be another mechanism that is involved in decreasing serum glucose level and improvement in insulin sensitivity.

The beneficial effect of exercise training on improved insulin sensitivity in STZ-induced diabetic rats could be partly mediated by changing the secretion of myokines [2]. One of these myokines is RBP4, which is suggested to be involved in the development of insulin resistance [2]. The results of this study showed that endurance exercise training reduces gastrocnemius RBP4 contents in STZ-diabetic rats. Intriguingly, it has been reported that a reduction in insulin resistance and serum RBP4 in spontaneously hypertensive rats following 10 weeks aerobic exercise training [19]. Besides, 7 weeks running on treadmill improved insulin sensitivity in diabetic rats by reducing RBP4 expression in extensor digitorum longus muscle, visceral and subcutaneous fat tissue [2]. Hence, a reduction in gastrocnemius RBP4 contents in STZ-diabetic rats may be attributed to reduction in RBP4 expression [2]. In contrast, other studies have reported no significant changes in RBP4 content, glucose, insulin, and HOMA-IR in healthy athletic women following 8 weeks aerobic exercise training [4, 6]. In addition, Mansouri et al. [2] reported that exercise training had no significant effect on muscle RBP4 content in healthy rats. Collectively, it is thought that exercise training had no significant influence on RBP4 content in healthy subjects.

Based on the above evidence, RBP4 reduces insulin-dependent glucose uptake in muscle tissue through reducing phosphoinositide-3-kinase activity and subsequent phosphorylation of the insulin receptor substrate-1 [12]. Apart from this, RBP4 resulted in downregulation of GLUT4 expression [19]. In this regard, a simultaneous reduction in gene expression of GLUT4 induced by 10 weeks running on treadmill has been attributed to increasing levels of RBP4 [19]. Furthermore, Graham et al. [11], in a review, demonstrated an inverse correlation between serum RBP4 levels and adipocyte GLUT4 protein. In fact, part of the improvement in insulin sensitivity in this study might be due to increased GLUT4 expression in muscle cells [19], so that the inhibitory effect of RBP4 has been removed from it. RBP4 increases the expression of phosphoenolpyruvate carboxylase in the liver, which eventually leads to increased hepatic glucose output that serves to raise blood glucose [11]. Furthermore, it has been suggested that RBP4 had negative effect on the secreting function of B cells [2, 24]. It has been demonstrated that elastic band exercise [17], resistance exercise training [16], and endurance running [5, 9] reduce serum RBP4 in diabetic [16, 17] and obese [5, 9] subjects. In addition, 7- to 10-week running on treadmill improved insulin sensitivity and reduced serum RBP4 in diabetic [2] and hypertensive [18, 19] rats. Hence, part of improved insulin sensitivity and reduced glucose levels in STZ-diabetic rats following exercise training in this study might be due to reduced serum RBP4, although serum concentrations of RBP4 were not measured in this study.

Conclusion

Moderate endurance exercise training through a reduction in gastrocnemius RBP4 content in STZ-diabetic rats reduces glucose levels, which subsequently improves insulin sensitivity.

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Funding sources: None.

Authors' contribution: M-RY and HT conceived the study and its design and coordination. Both authors were involved in the data collection, data analysis, and drafting of the manuscript. Both authors read and approved the final version of the manuscript, and agreed with the order of presentation of the authors.

Conflict of interest: There are no conflicts of interest.

Acknowledgement: The authors would like to thank the staff of the animal laboratory at the Ilam University of Medical Sciences for their valuable assistance in carrying out the exercise protocols and animal surgery.

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