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ORIGINAL ARTICLE

Effect of endurance training on retinol-binding protein 4 gene expression and its protein level in adipose tissue and the liver in diabetic rats induced by a high-fat diet and streptozotocin

Masoume Mansouri[1,2], Rohollah Nikooie[3], Abasali Keshtkar[4], Bagher Larijani[2], Kobra Omidfar[5]*

[1]Obesity and Eating Habits Research Center, [5]Biosensor Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, [2]Endocrinology and Metabolism Research Center, [4]Osteoprosis Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, and [3]Department of Exercise Physiology, Faculty of Physical Education and Sport Science, Shahid Bahonar University, Kerman, Iran

Keywords
Endurance training, Insulin resistance, Retinol-binding protein 4 gene and protein expression

***Correspondence**
Kobra Omidfar
Tel.: +98-21-88220037-38
Fax +98-21-88220052
E-mail address: omidfar@tums.ac.ir

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ABSTRACT

Aims/Introduction: The present study was designed to investigate from which tissues the decrease in retinol-binding protein 4 (RBP4) expression could contribute to the improvement of serum RBP4 and insulin resistance (IR) after endurance training.

Materials and Methods: Male 7-week-old Wistar rats were randomly assigned into four groups including control (C), trained (T), diabetic control (DC) and trained diabetic (TD). At 8 weeks-of-age, diabetes was induced by a high-fat diet and intraperitoneal injection of low-dose streptozotocin (STZ; 35 mg/kg). Rats in the T and TD groups carried out a 7-week exercise program on a motorized treadmill (15–20 m/min for 20 min/day for 5 weeks), whereas the C and DC remained sedentary in their cages. Tissues gene expression and protein levels of RBP4 were assessed by using real-time polymerase chain reaction and western blot, respectively, while serum RBP4 was measured using an enzyme-linked immunosorbent assay kit.

Results: Exercise significantly improved IR and reduced serum concentration of RBP4 in the TD group. This reduction of serum RBP4 was accompanied by decreased RBP4 protein expression in visceral fat tissue. In contrast, exercise had no significant effect on RBP4 expression in liver and subcutaneous fat tissue in the TD group. Exercise also significantly decreased RBP4 gene expression in visceral fat tissue and muscle, whereas the effect of exercise on liver RBP4 messenger ribonucleic acid expression was not significant.

Conclusions: The present study showed that the mechanism for RBP4 reducing the effect of endurance training could involve decreased RBP4 messenger ribonucleic acid expression and its protein level in adipose tissue in STZ-induced diabetic rats.

INTRODUCTION

The beneficial effect of exercise training on reduced insulin resistance in streptozotocin (STZ)-induced diabetes[1] could be partly mediated by changing the secretion of adipokines. One of these adipokines is retinol-binding protein 4 (RBP4), which is suggested to be involved in the development of insulin resistance[2–7]. Based on the evidence, increased serum RBP4 concentration reduces insulin-dependent glucose uptake by muscle tissue through reducing phosphoinositide-3-kinase (PI-3-kinase) activity and subsequent phosphorylation of the insulin receptor substrate-1 (IRS-1), which are necessary components of the insulin signaling pathway[2]. In contrast, in the liver, RBP4 increases the expression of the enzyme, phosphoenolpyruvate carboxylase (PEPCK), which eventually leads to increased hepatic glucose output that serves to raise blood glucose[3]. In addition, a negative effect of RBP4 on the secreting function of β-cells has been suggested[6,8]. Some studies consider the impact of exercise on insulin

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resistance and serum RBP4. The results of these studies showed that the insulin sensitizing effect of exercise was accompanied with reduced RBP4 concentration. A significant decrease of serum RBP4 and an improvement of insulin sensitivity were observed in a wide range of subjects with insulin resistance[3]. Similar findings have also been observed in obese children after exercise[9]. However some studies reported that normalization of insulin sensitivity was not mediated by changes in serum RBP4 in an exercised animal model[10]. Although serum RBP4 seems to be decreased by exercise in most cases, some studies could not find any association between RBP4 and insulin resistance[10,11]. Therefore, further investigation of the effect of exercise on insulin resistance and serum RBP4 is still necessary. In addition, the effects of exercise on RBP4 expression in organs, such as the liver and adipose tissue, have not been reported and it is not clear from which tissue decrease in RBP4 expression could contribute to the improvement of serum RBP4 and insulin resistance after endurance training. Thus, in the present study, we investigated whether endurance exercise could change circulating RBP4 in STZ-induced diabetic rats, and whether these changes would be accompanied by changes in RBP4 messenger gene expression and its protein level in the liver, adipose tissue and muscles. Finally, we investigated in which tissue the degree of changes in RBP4 expression was higher.

MATERIALS AND METHODS

Animals

Male 5-week-old Wistar rats were purchased from the Pasteur Institute (Tehran, Iran), and animals were maintained in an air-conditioned room (temperature 22 ± 3°C) with a 12-h light–dark cycle. All rats were fed chow diet and water *ad libitum* during the 2-week acclimation period while their bodyweights were monitored daily. At the age of 7 weeks, the rats were randomly assigned to four groups including controls (C), trained (T), diabetic (DC) and trained diabetic (TD). The protocol of this experimental study was approved by the Ethical Committees on Animal Care at the Endocrinology and Metabolism Research Center of Tehran University of Medical Sciences.

Diabetes Induction Method

At the beginning of the rats' eighth week of age, all diabetic rats were fed a high-fat diet (Table 1) for 2 weeks, and then diabetes was induced by intraperitoneal injection of low-dose STZ (35 mg/kg) dissolved in citrate buffer (0.01 mol/L, pH 4.5). Blood samples were obtained from the orbital sinus and analyzed for non-fasting glucose. Blood was collected through a heparinized tube and plasma separation was carried out by centrifuge at 3,000 *g* for 10 min at 4°C. Plasma glucose concentration was determined by the glucose oxidase method using an autoanalyzer[12] (Hitachi, Hitachi, Japan). Animals with non-fasting plasma glucose level of ≥300 mg/dL were considered diabetic, then housed in a separate cage and fed a high-fat diet (HFD) for 7 weeks. The rats from the C and T groups were fed a conventional pellet diet.

Table 1 | Composition of high fat diet

Ingredients	Diet (g/kg)
Powdered NPD	365
Fat	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
DL-methionine	03
Yeast powder	01
Sodium chloride	01

NPD, normal pellet diet.

Training Intervention

Exercise training was started at 10 weeks-of-age. Endurance training was carried out every day for 7 weeks. Initially, the T and TD groups were familiarized with a motor-driven treadmill running at low speeds (15–20 m/min) for 20 min/day for the first 5 days. Thereafter, the duration was increased gradually over the 7-week period, until the animals were running for 35 min/day at 30 m/min for the last 2 weeks. Electrical shock was not used to force the rats to run. The C and D rats remained sedentary in their cages for the duration of the 7-week training program.

Collection of Blood and Tissue Samples

At the end of the experimental period, the animals were fasted overnight, but they were allowed to take water. The next morning, animals were anesthetized (ketamin [90 mg/kg] and xylazin [10 mg/kg]), and the soleus and extensor digitorum longus (EDL) muscles, visceral and subcutaneous fat, and liver were excised rapidly, frozen in liquid nitrogen and stored at −80°C for further analysis. Blood samples were withdrawn by heart puncture, plasma or serum separated as aforementioned and stored at −80°C. These samples were used for measurement of fasting blood glucose, fasting plasma insulin, fasting serum RBP4 concentration and plasma lipid profile.

Insulin Resistance Confirmation

Type 2 diabetic rats were selected according to the following insulin resistance criteria: (i) fasting plasma insulin level above 60 pmol/L; and (ii) homeostasis model assessment of insulin resistance >2.5[13].

Biochemical Measurement

Plasma glucose was determined by the glucose oxidase method[12]. Serum RBP4 was quantitatively measured by enzyme-linked immunosorbent assay (cat: RB0642EK; AdipoGen Inc., Seoul, Korea). The sensitivity of the assay was 60 pg/mL, and the intra-assay and interassay coefficients were lower than 10%. Plasma insulin was measured by mouse/rat insulin enzyme-linked immunosorbent assay kit (cat: EZRMI-13K; Millipore, Billerica, MA, USA). The sensitivity of the assay was

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0.1 ng/mL, and the intra-assay and interassay coefficients were lower than 10%. Total cholesterol and triglycerides were determined by standard enzymatic procedures using an autoanalyzer (Hitachi).

Western Blotting

Approximately 150–200 mg of each tissue was powdered with a cold mortar and pestle in liquid nitrogen. Muscle and liver were homogenized in 1 mL of phosphate-buffered saline (PBS) and centrifuged at 12,000 *g* for 15 min at 4°C, and supernatant was removed. Fat samples were lysed in Tris buffer (20 mmol/L Tris, 1% NP-40; 137 mmol/L NaCl; 1 mmol/L CaCl2; 1 mmol/L MgCl2; 10% (v/v) glycerol; 1 mmol/L dithiothretol; 1 mmol/L phenylmethanesulphonyl fluoride; 2 mmol/L Na3VO4). Total protein was determined by the Bradford method using bovine serum albumin (BSA) as a standard. A total of 20 μg total protein of each sample was loaded and separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred by electroblotting onto polyvinylidenedifluoride (PVDF) membranes (Amersham, Pharmacia Biotech, Upsala, Sweden). Membranes were incubated for 1 h at room temperature on an orbital shaker in blocking solution (150 mmol/L NaCl, 0.1% Tween 20 and 50 mmol/L Tris, pH 7.5 [TTBS], 5% skimmed milk) and incubated in primary antibody overnight at 4°C in Tris-buffered saline. Membranes were washed once for 15 min and then twice for 5 min in TTBS, and then incubated for 90 min at room temperature with secondary antibody in Tris-buffered saline. Membranes were washed as aforementioned, and protein expression was then detected by enhanced chemiluminescence according to the manufacturer's instructions. Autoradiographic film was exposed to membranes and developed. Molecular weight standards were used to identify appropriate antibody binding. Band densities were determined with image densitometer software. Rat RBP4 protein was used as a positive control and to fix an arbitrary unit to allow comparison between experiments (1 equals the RBP4 signal generated by 5 ng of RBP4 protein).

Real Time Polymerase Chain Reaction

Tissue was powdered with a cold mortar and pestle, and total ribonucleic acid (RNA) was isolated using Isol RNA-Lysis reagent. Approximately 50 mg of powdered tissue was added to 1 mL ice-cold Isol and homogenized. Homogenates were centrifuged at 12,000 *g* for 10 min at 4°C to remove the pellet. Chloroform (200 μL) was added to the supernatant fraction and shaken vigorously for 15 s. The organic and aqueous phases were separated by centrifugation at 12,000 *g* for 15 min. The aqueous phase was removed and 600 μL isopropanol was added, and RNA was isolated according to the manufacturer's instructions. RNA concentration and purity was estimated by OD 260/280.

Complementary deoxyribonucleic acid (cDNA) synthesis was carried out with 1 μg RNA in a total reaction volume of 20 μL using random hexamer oligonucleotides. Reverse transcriptase reactions were carried out according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (PCR) was carried out using a 7300 Real Time PCR System (Applied Biosystems, Step One, Weiterstadt, Germany). The PCR reaction was carried out using Syber Green II, and ROX was used as a reference dye. The concentration of each primer and cDNA were 100 pm and 100 ng, respectively. The thermocycling conditions were: 10 min at 95°C, followed by 40 cycles at 95°C for 15 s and 60°C for 60 s. Table 2 shows the primer sequences used for real-time PCR.

Table 2 | Primer sequences used for real-time polymerase chain reaction analysis

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Size (bp)
RBP4	GGTGAGCAGCTTCAGAGTC	ACCATGTCTGCACACACTTC	204
18S	GTTGGTTTTCGGAACTGAGGC	GTCGGCATCGTTTATGGTCG	206

Gene expressions were expressed relative to the expression of the 18S housekeeping gene. To avoid detection of non-specific PCR products, the purity of each amplified product was confirmed using a melting curve analysis. Data quantification was carried out using the $2^{-\Delta\Delta CT}$ method[14]. Primer amplification efficiencies were determined using serial cDNA dilutions, and were determined to be approximately equal.

Statistical Analysis

All data are shown as means ± standard deviation. The differences were considered to be significant if $P \leq 0.05$. Comparisons of variables between study groups were carried out by using one-way analysis of variance (ANOVA) test. When a significant effect was found, *post-hoc* Scheffé analysis was carried out to determine which mean differences were significant. All analysis was carried out using SPSS version 16 (SPSS, Chicago, IL, USA).

RESULTS

Induction of diabetes caused a significant increase in bodyweight, fasting glucose, insulin and homeostasis model assessment index. Diabetes also resulted in increased levels of triglyceride and cholesterol (Figure 1, Table 3). Exercise caused a significant decrease in bodyweight in the TD group. Fasting insulin and glucose were significantly lower in the TD group compared with the D group; however, they were still significantly higher compared with the C group. Lipid profile also significantly decreased in the TD group. The values of lipid profile in the TD group were still much higher than those in the C and T groups (Table 3).

Change of Serum Concentration of RBP4 and RBP4 Protein Expression

Diabetes induced a significant increase in serum level of RBP4 (almost a threefold increase in the DC group compared with

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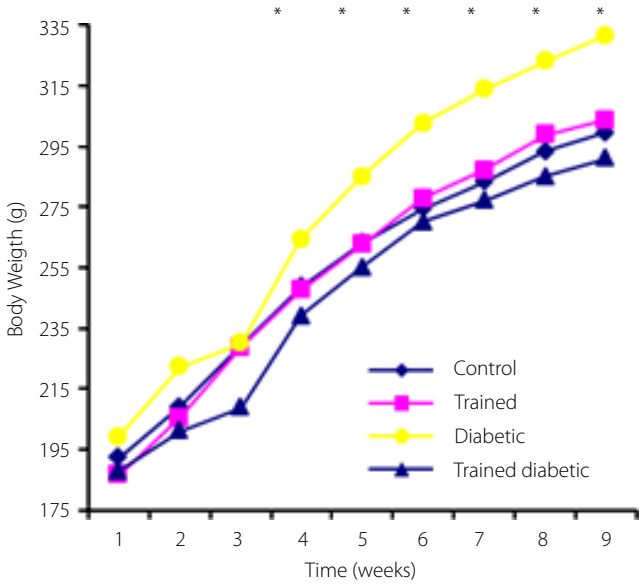

Figure 1 | Weekly bodyweight changes during the course of the study among four experimental groups (n = 6 rats in each group)

the C group). RBP4 expression in the liver, adipose tissues and muscle were significantly higher in the DC group than the other groups. Among them, the liver showed the highest increase in RBP4 expression (almost a 3.5-fold increase in DC compared with the C group; Table 4). Exercise led to a reduction in serum concentration of RBP4 in the T group. However, a significantly higher reduction occurred in the TD group (Figure 2).

Exercise also significantly decreased RBP4 protein expression in visceral fat tissue in the TD group compared with the diabetic group. However, these reductions did not reach the levels of the C group. In contrast, exercise had no significant effect on RBP4 expression in the liver, muscle and subcutaneous fat tissue in the TD group (Table 4).

RBP4 Gene Expression

Diabetes induced RBP4 gene expression in adipose tissue, the liver and muscle. The highest increase was observed in visceral fat and the liver, respectively, whereas the muscle showed the lowest reduction (Figure 3). These findings were consistent with RBP4 expression in the liver, fat tissues and muscle.

Exercise significantly decreased RBP4 gene expression in fat tissues, whereas the highest change was observed in visceral fat. Exercise also induced a significant change in muscle RBP4 expression. However the effect of exercise on liver RBP4 mRNA expression was not significant (Figure 3).

DISCUSSION

In the present study, we tried to answer the question: "reduction in which of the three tissues, liver, adipose tissue or muscle RBP4 expression" will be accompanied with a decrease in serum RBP4 concentration in response to endurance training in type 2 diabetic rats?"

In addition, simultaneous measurement of RBP4 expression in the three aforementioned tissues was carried out to investigate which tissue is a predominant source of serum RBP4 in the insulin resistant state.

It is notable that this is the first report showing the differential expression pattern of RBP4 between adipose tissue, liver and muscle in response to exercise training.

In the present study, the rat model for type 2 diabetes was developed by combining a HFD, which produced insulin resistance, and a low dose of STZ injection, which caused the initial β-cell dysfunction. This kind of rat model could closely simulate the metabolic disturbances that occur in type 2 diabetes[15]. Thus, this model could be suitable for evaluating the effect of endurance exercise (which acts through modifying insulin resistance and serum RBP4) on mRNA RBP4 and its protein expression in different tissues. In addition, the fat we used to feed the rats in this experiment was tallow, which mainly consists of saturated (43%), monounsaturated fatty acids (50%) and polyunsaturated fatty acids (5%)[16]. The present findings showed that induction of diabetes caused a significant increase

Table 3 | Effect of 7-week endurance training on bodyweight and biochemical parameter

Variable	Control (n = 6)	Trained (n = 6)	Diabetic control (n = 6)	Trained diabetic (n = 6)
Weight (g)	299.79 ± 30.81*	303.82 ± 26.06*	331.7 ± 18.14	291.17 ± 25.21*
Amount of food intake (g/kg weight)	16.8 ± 2.5	14.2 ± 3.1	13.7 ± 2.7	13.8 ± 3.1
Fasting glucose (mg/dL)	106.4 ± 9.26	101.62 ± 9.02*	394.44 ± 48.74	301.7 ± 47.95*†
Fasting insulin (nmol/L)	6.25 ± 1.15	5.73 ± 0.68	10.55 ± 1.12	8.95 ± 0.78*†
HOMA-IR index	1.62 ± 0.24*	1.45 ± 0.19*	10.48 ± 1.44	6.68 ± 1.31*†
Triglyceride (mg/dL)	33.8 ± 4.4*	35.7 ± 7.1*	114.6 ± 12.4	46.14 ± 5.1*
Cholesterol (mg/dL)	48.4 ± 5.3*	43.5 ± 6.07*	108.3 ± 10.1	47.6 ± 9.1*

Values presented as mean ± standard deviation. *Significant differences with diabetic control. †Significant difference with control. HOMA-IR, homeostasis model assessment of insulin resistance.

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Table 4 | Effect of 7-week endurance training on retinol-binding protein 4 protein and gene expression in different tissues

	Control (n = 6)	Trained (n = 6)	Diabetic control (n = 6)	Trained diabetic (n = 6)	DC *vs* C (P)	DC *vs* TD (P)
Serum RBP4 (ng/mL)	3.52 ± 0.34	3.23 ± 0.27	10.22 ± 1.02	6.49 ± 0.69	0.000	0.000
Liver RBP4	2.43 ± 0.37	2.47 ± 0.23	8.38 ± 0.83	7.91 ± 0.67	0.000	0.59
Visceral fat RBP4	1.53 ± 0.32	1.44 ± 0.19	3.10 ± 0.34	2.50 ± 0.27	0.000	0.015
SC fat RBP4	1.35 ± 0.18	1.24 ± 0.15	2.47 ± 0.21	2.35 ± 0.34	0.000	0.85
EDL RBP4	0.41 ± 0.09	0.36 ± 0.06	0.93 ± 0.15	0.88 ± 0.21	0.000	0.9
Liver RBP4 mRNA	1.00	0.86 ± 0.30	2.33 ± 0.56	2.27 ± 0.41	0.000	0.10
Visceral fat RBP4 mRNA	1.00	0.69 ± 0.19	2.37 ± 0.53	1.31 ± 0.25	0.000	0.000
SC fat RBP4 mRNA	1.00	0.92 ± 0.41	1.83 ± 0.45	1.14 ± 0.31	0.005	0.02
EDL RBP4 mRNA	1.00	0.64 ± 0.13	2.04 ± 0.53	1.34 ± 0.45	0.000	0.000

Values presented in mean ± standard deviation. P-value represents significant level between groups differences based on ANOVA with *post-hoc* Scheffé's test. C, control; DC, diabetic control; EDL, extensor digitorum longus; mRNA, messenger ribonucleic acid; RBP4, retinol-binding protein 4; SC fat, subcutaneous fat; TD, trained diabetic.

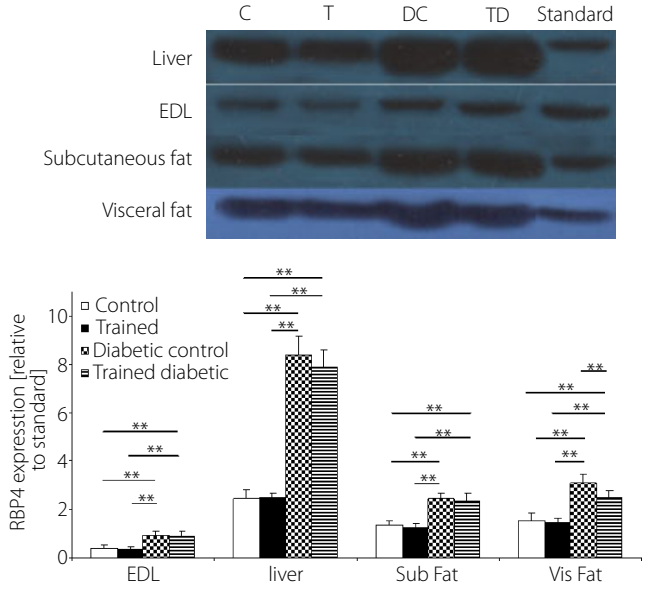

Figure 2 | The effect of 7-week endurance training on retinol-binding protein 4 (RBP4) protein expression (relative to standard) determined by western immune blot in different tissues including muscle (extensor digitorum longus [EDL]), liver, subcutaneous fat (subfat) and visceral fat (visfat) at the end of the study. Data are presented for each group as means ± standard deviation (n = 6 rats in each group). *P < 0.05, **P < 0.01.

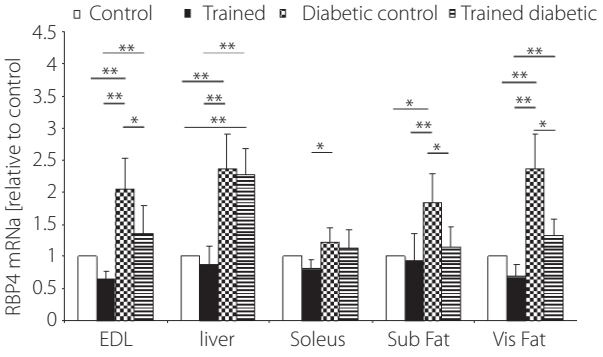

Figure 3 | The effect of 7-week endurance training on retinol-binding protein 4 (RBP4) messenger ribonucleic acid expression (relative to control) determined by real-time polymerase chain reaction in different tissues including muscles, liver, subcutaneous fat (subfat) and visceral fat (visfat) at the end of the study. Values are means ± standard deviation (n = 6 rats in each group). *P < 0.05, **P < 0.01.

in bodyweight. In contrast, endurance training normalized bodyweight and improved lipid profile. We believe improvement of mitochondrial function and increased energy expenditure in different tissues is one of the mechanisms[17,18] by which the 7-week endurance exercise caused these changes in trained diabetic rats.

We found that induction of diabetes significantly enhanced insulin resistance and serum RBP4. These increases are accompanied with increased RBP4 expression at both a transcriptional and translational level in the liver, adipose tissues and muscle. The increased serum level of RBP4 in diabetic rats might be a consequence of its increased mRNA and protein expression in both the liver and adipose tissues, with the highest expression level in the liver followed by the second rate in the adipose tissues. Thus, in the present study, increased circulating RBP4 (10.21 in DC *vs* 3.52 in C, P < 0.000) could be mediated by increased RBP4 mRNA expression and its protein level mainly in the liver (Table 2).

This finding is supported by a study that reported RBP4 is predominantly expressed in the liver in rodents and rats[19]. However, our findings, which showed that adipose tissue is not the main organ to produce RBP4 in an insulin resistant state, are inconsistent with those showing that visceral fat is the major source of RBP4 production in an insulin resistant state and type 2 diabetes[2,9,20–22].

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One possible explanation could be related to the rat model we developed in the present study. Although this diabetic model was closely similar to the metabolic characteristics of type 2 diabetes, the question remains as to whether the RBP4 tissue expression pattern in an insulin resistant state as a result of induction of diabetes is still completely similar to real type 2 diabetes. Endurance exercise significantly improved insulin resistance, and this reduction was accompanied by dramatically reduced RBP4 in the circulation in diabetic trained rats compared with diabetic rats (10.22 *vs* 6.49, $P < 0.000$). These results support previous findings reporting interventions as the cause for reduction in serum RBP4[2,3,9,23], and suggesting that a decrease of serum RBP4 could be one of the mechanisms underlying the improvement of insulin resistance with exercise[24]. However, apart from RBP4, exercise also changed the serum level of other adipokines, such as adiponectin and leptin, which influence insulin resistance[24]. Perhaps measuring these adipokines in comparison with RBP4 would have given us more information in this regard. Therefore, the findings presented here should be considered with this limitation.

The most important and novel finding of the present study was that the 7-week exercise resulted in a significant reduction in RBP4 expression in visceral adipose tissue of HFD–STZ induced diabetic rats. This reduction was accompanied by a parallel decrease in RBP4 expression in this tissue and serum RBP4 concentration. In contrast, endurance training was unable to change RBP4 mRNA level and its protein expression in the liver of diabetic rats. Thus, the effect of exercise on RBP4 protein expression was specific to visceral fat tissue and did not occur in the liver. These findings suggest that decreased adipose tissue RBP4 expression after endurance training might cause a reduction in serum RBP4, which in turn might cause a decrease in insulin resistance through different reported pathways[24]. It could be speculated that one mechanism by which endurance exercise reduced RBP4 expression in visceral fat tissue might be related to the reduced adipocyte size and fat mass, which was not measured in the present study. Consistent with our suggestion, previous studies showed that weight loss and exercise led to reduced adipocyte size, which in turn is an important determinant for the secretion of several adipokines[25–27].

As the liver is the major tissue to produce and secrete RBP4, it was expected that endurance training should decrease liver RBP4 expression. However, this effect was not observed in the present study. One possible explanation for this finding is that the time and duration of the exercise session might not have been sufficient to induce significant changes in liver RBP4 protein expression. Based on the literature, the training effect is tissue specific, and any adaptive response to exercise training depends on the degree of involvement of tissue during exercise. As the liver is an inactive tissue during exercise, its involvement in exercise is limited to long duration workouts, and takes over 90 min to produce energy through the gluconeogenesis process. Because our exercise session took 40 min, the liver might not have been influenced. In contrast, visceral tissue is very active during this endurance exercise, in which the major substrate for energy supply is free fatty acid with two primary sources of visceral and subcutaneous fat tissues[28]. Furthermore, recent reports have shown that elevated serum RBP4 is closely associated with fatty liver[29,30]. Increased plasma RBP4 level might be a consequence of fat accumulation in the liver as a result of the induction of diabetes, and continuation of a HFD in diabetic rats and this period of exercise is not enough to reverse this metabolic disturbance. Our suggestion was supported by Vieira *et al.*[31] who showed that just 12 weeks of exercise could completely eliminate hepatic esteatosis regardless of continuing the HFD. Thus, it might be possible that changes in liver RBP4 expression might present themselves during a longer exercise period. In contrast to diabetic rats, training did not change serum RBP4 concentration in trained rats. In other words, we showed that RBP4 levels were decreased after exercise only in the rats with a higher basic level of RBP4. This finding suggests that the influence of exercise on serum RBP4 concentration is dependent on its initial levels. It is conceivable that the basal RBP4 level in trained diabetic rats, but not in trained animals, might be high enough to be changed by 7 weeks of exercise training. This is also supported by other studies that showed that the effect of exercise seems to be more evident in those with high initial RBP4[2,32]. Along with this evidence, in a previous study we also showed that the basic level of RBP4 was the only predictor of the after-exercise plasma RBP4 concentration[33].

Although our 7-week endurance training significantly improved insulin resistance and reduced serum RBP4, it did not fully reverse them. In addition, mRNA RBP4 and its protein level in adipose tissue of trained diabetic rats did not return to the level of control rats. We suggest that the protective effect of exercise on adipose tissue RBP4 expression and serum RBP4 might be mediated through a reduction in visceral fat or reduction in adipose tissue inflammation. It has been reported that serum RBP4 was significantly correlated with visceral fat mass and visceral adipose RBP4 expression[34]. In addition, a decrease in serum RBP4 level achieved by exercise training predicts the improvement in insulin sensitivity[35]. Thus, the degree of reduction of body fat mass and adiposity, which was not measured in the present study, might be important in this regard. We assumed that the second mechanism by which exercise reduces adipose tissue RBP4 expression and serum RBP4 might involve improvement in the inflammation induced by diabetes and a HFD. This assumption was supported by evidence that showed a close connection between RBP4 expression and its protein level, and inflammatory markers[36–41].

In addition, recent studies confirmed that the period of exercise intervention is a determinant factor in reducing inflammatory markers expression induced by a long-term HFD[10,42]. We suggest that under the condition of a HFD, the impact of endurance training on RBP4 expression was moderate. Thus, a longer period of exercise program might be necessary to completely reverse the effect of continued consumption of HFD

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and induction of diabetes on mRNA RBP4 expression and its protein level in visceral fat tissue of diabetic mice in this experiment. Training intervention also resulted in a significant decrease in RBP4 mRNA level in subcutaneous fat tissue and muscle. However, the degree of changes in RBP4 mRNA expression was much higher in visceral fat than other tissues in the trained diabetic group. In this regard, the reduction in RBP4 mRNA in subcutaneous fat tissue and muscle was not reflected at the RBP4 protein level in these tissues. In other words, exercise induces muscle and subcutaneous tissues to decrease RBP4 mRNA expression without a decrease in its protein level. Several unknown regulatory mechanisms might be a reason for affecting the post-transcriptional of RBP4[43]. Furthermore, according to previous reports, gene expression levels do not necessarily correspond to protein expression[44]. Finally, the current study showed that 7-week endurance training was able to reduce insulin resistance and serum RBP4, confirming its potential effect on RBP4 mRNA and protein expression in visceral fat tissue.

In conclusion, we found that the RBP4-reducing effect of endurance training is predominantly mediated by reduction of RBP4 mRNA expression and its protein level in adipose tissue in diabetic rats induced by HFD and streptozotocin. In fact, the beneficial effect of 7-week endurance training on insulin resistance and serum RBP4 might not be reflected by changes in RBP4 mRNA expression and its protein level in the liver.

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