

Effects of exercise intensity on RBP4 levels in female athletes

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Abstract

Introduction: Retinol-binding protein 4 (RBP4) has been described as an adipokine that contributes to insulin resistance, obesity and the metabolic syndrome. The aim of present study was to examine the effects of exercise intensity on RBP4 levels in female athletes.

Material & Methods: 30 female karate athletics were randomly assigned to one of the High intensity training group (HIT, n=10), moderate intensity training group (MIT, n=10), or control group (n=10). The HIT training group performed endurance training 3 days a week for 8 weeks at an intensity corresponding to 75-80% individual maximum oxygen consumption for 45 min. The MIT group performed endurance training at the same days, times and duration at an intensity corresponding to 50-60% individual maximum oxygen consumption for 45 min.

Results: Body mass and BMI increased ($P < 0.05$) after 8 weeks HIT and MIT compared to the control group. For body fat percentage and maximal oxygen consumption there were no significant differences between the exercise groups and the control group. There were virtually no changes in body fat percentage, fasting glucose, insulin, insulin resistance and RBP4 levels after 8 weeks HIT and MIT.

Conclusions: Serum RBP4 levels were not affected by 8 weeks HIT and MIT in female athletes.

Key words: Exercise intensity, RBP4, Female athletes, Lipocalin

1. Introduction

Retinol binding protein 4 (RBP4), is a human lipocalin family that in humans is encoded by the RBP4 gene (1,2). RBP4 is a newly discovered fat derived adipokine that specifically binds to retinol (3) and transthyretin (4). RBP4 has recently been added to the list of adipokines that may link obesity with insulin resistance and type 2 diabetes (5,6). In fact, elevated serum RBP4 levels were associated with the components of metabolic syndrome in insulin-resistant subjects (6). Transgenic over expression of RBP4 or injection of recombinant RBP4 decreases insulin sensitivity in normal mice. In contrast, normalization of RBP4 levels in obese mice restores insulin sensitivity (5). In addition, Seo et al. (2008) demonstrated an association between RBP4 levels and non-alcoholic fatty liver disease (7).

Exercise has been shown to have beneficial effects on obesity, type 2 diabetes, and the metabolic syndrome. Several studies exploring the effects of exercise on circulating RBP4 levels have resulted inconsistent findings. Circulating RBP4 not affected (8,9), decreased (10,11) or increased (12) in response to exercise. For example, Cho et al. (2006) reported that there was no significant change in RBP4 levels in obese women after 12 weeks moderate exercise training (8), while Lim et al. (2008) indicated that RBP4 levels decrease after 10 weeks exercise in middle-aged women (10). The effect of exercise intensity on RBP4 level is not well known; therefore,

the effects of 8 weeks of high intensity training (HIT) and moderate intensity training (MIT) was investigated in the present study.

2. Materials and Methods

Subjects

30 female karate athletes (24.1 ± 4.3 years; mean \pm SD) participated in this study. All the subjects were asked to complete a personal health and medical history questionnaire, which served as a screening tool. The subjects were given both verbal and written instructions outlining the experimental procedure, and written informed consent was obtained. Our participants were nonsmokers and none of them had any disease. All the subjects completed the 3-day diet recall forms and were instructed to maintain their normal physical activity and dietary habits throughout the study. The subjects were randomly assigned to one of the HIT group (n=10), MIT group (n=10), or control group (n=10). The study was approved by the Islamic Azad University, Fars Science & Research branch Ethics Committee.

Exercise Training

The subjects in the HIT and MIT group were performed 8 weeks aerobic exercise 3 sessions per week. The intensity of exercise was customized for each subject based on the relationship between heart rate and oxygen uptake measured at baseline. During the 8 weeks intervention, the subjects in the MIT group and HIT group were trained for 45 min per session at a heart rate corresponding to 50-60% and 75-80% of the maximal oxygen uptake measured at baseline respectively. Each participant wore a heart rate monitor (Beurer, PM70, Germany) to ensure accuracy of the exercise level. Subjects performed the exercise training besides their karate training of team.

Measurements

Anthropometric and body composition measurements

Height and body mass were measured, and body mass index (BMI) was calculated by dividing body mass (kg) by height (m^2). Waist circumference was determined by obtaining the minimum circumference (narrowest part

of the torso, above the umbilicus) and the maximum hip circumference while standing with their heels together. The waist to hip ratio (WHR) was calculated by dividing waist (cm) by hip circumference (cm) (13). Body fat percentage was assessed by skinfold thickness protocol. Skinfold thickness was measured sequentially, in triceps, suprailiac, and thigh by the same investigator using a skinfold caliper (Harpenden, HSK-BI, British Indicators, West Sussex, UK) and a standard technique (13).

Measurement of VO_{2max}

VO_{2max} was determined by Rockport One-Mile Fitness Walking Test. In this test, an individual walked 1 mile as fast as possible on a track surface. Total time was recorded and HR was obtained in the final minute (13). VO_{2max} was calculated using formula (13).

Biochemical analyses

Fasted, resting morning blood samples (10 ml) were taken at the same time before and after 8 weeks intervention. For menstrual status, all the participants were menstruating regularly and defined as eumenorrheic (28- to 32-day menstrual cycles during the previous year); all testing was performed during the follicular phase of the menstrual cycle. All the subjects fasted at least for 12 hours and a fasting blood sample was obtained by venipuncture. Serum obtained was frozen at -22°C for subsequent analysis. The serum RBP4 level was measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) kits (Casabio Biotech Co. LTD.; China). The sensitivity of kit was $0.1\ \mu\text{g}/\text{ml}$. Serum glucose was determined by the enzymatic (GOD-PAP, Glucose Oxidase-Amino Antipyrine) colorimetric method (Pars Azmoun, Tehran, Iran). The intra and inter-assay coefficients of variation for glucose were $<1.3\%$ and a sensitivity of $5\ \text{mg}/\text{dl}$. The serum insulin level was measured by an electrochemiluminescence immunoassay (ECLIA) and the insulin resistance index was calculated according to the homeostasis model assessment (HOMA-IR) which correlates well with the euglycemic hyperinsulinemic clamp in people with diabetes (14).

Statistical analysis

Results were expressed as the mean \pm SD and distributions of all variables were assessed for normality. Data were analyzed using One-Way ANOVA test. The level of significance in all statistical analyses was set at $P < 0.05$. Data analysis was performed using SPSS software for windows (version 17, SPSS, Inc., Chicago, IL).

3. Results

Physical and physiological characteristics of the subjects at baseline and after training are presented in Table 1. Before the intervention, there were no significant differences in any of variables among the three groups. Body mass and BMI increased ($P < 0.05$) after 8 weeks HIT and MIT compared to the control group. For body fat percentage and maximal oxygen consumption there were no significant difference between the exercise groups and the control group.

Table 1. Anthropometric and metabolic characteristics (mean \pm SD) of the subjects before and after training

	Control group (n=10)		MIT group (n=10)		HIT group (n=10)	
	Pre	Post	Pre	Post	Pre	Post
Body mass (Kg)	57.8 \pm 8.4	57.9 \pm 8.4.0	55.1 \pm 9.1	57.4 \pm 9.1*	56.8 \pm 8.3	58.1 \pm 8.4*
BMI (Kg/m ²)	21.4 \pm 3.0	21.4 \pm 3.0	21.3 \pm 2.8	22.2 \pm 2.6*	21.2 \pm 2.9	21.7 \pm 2.9*
Body fat (%)	17.3 \pm 5	17.8 \pm 5.2	18.6 \pm 3.3	19.0 \pm 3.0	16.8 \pm 2.7	16.8 \pm 2.6
WHR	0.74 \pm 0.05	0.75 \pm 0.05	0.73 \pm 0.04	0.7 \pm 0.03	0.73 \pm 0.05	0.71 \pm 0.03*
VO _{2max} (ml.Kg.min ⁻¹)	47.7 \pm 3.5	47.9 \pm 3.4	47.5 \pm 3.8	48.2 \pm 2.8	49.7 \pm 2.8	51.2 \pm 3.6

*: $P < 0.05$ for between-group differences.

†: $P < 0.05$, pre *vs.* post training values.

RBP4, fasting glucose, fasting insulin, and HOMA-IR did not change in the exercise training groups compared with the control group (Table 2). No significant relationships were observed between RBP4 with body mass,

BMI, body fat percentage and HOMA-IR at baseline and after 8 weeks exercise.

Table 2. Biochemical characteristics (mean \pm SD) of the subjects before and after training

	Control group (n=10)		MIT group (n=10)		HIT group (n=10)	
	Pre	Post	Pre	Post	Pre	Post
FBS (mg/dl)	90.3 \pm 8.8	85.8 \pm 8.5	89.1 \pm 2.7	84.6 \pm 7.9	88.8 \pm 11.1	86.4 \pm 13.1
Insulin (μ U/ml)	8 \pm 2.9	9.6 \pm 4	10.2 \pm 4.1	10.1 \pm 2.3	10.4 \pm 7.4	11.2 \pm 7.5
HOMA-IR	1.7 \pm 0.5	1.9 \pm 0.7	2.2 \pm 0.9	2 0.5	2.5 \pm 1.6	2.4 \pm 1.7
RBP4 (μ g/ml)	11.6 \pm 4.1	12.5 \pm 3.3	12.5 \pm 3.4	12.9 \pm 3.9	11.1 \pm 3.2	12 \pm 1.9

4. Discussion

RBP4, was identified as novel adipokines associated with obesity, type 2 diabetes and the metabolic syndrome (5,6,15). The effects of exercise intensity on serum RBP4 is still unclear. The purpose of this study was to examine the effects of 8 weeks HIT and MIT on serum RBP4 levels in female athletes. Our results showed that RBP4 levels at baseline were lower in female athletes (median 4.1 [range 3.7–16.7 μ g/ml]) than in impaired glucose tolerance, type 2 diabetic and healthy subjects. Cho et al. (2006) demonstrated median levels of plasma RBP4 concentrations in the impaired glucose tolerance (median 18.9 [range 11.2–45.8 μ g/ml]), type 2 diabetic patients (median 20.9 [range 9.9–48.5 μ g/ml]), and healthy subjects (median 18.1 [9.3–30.5 μ g/ml]) (16). Thus it seems that playing karate improves levels of RBP4 in females.

Our results are in agreement with previous reports showing that there was no relationship between RBP4 levels and body composition parameters including body mass, BMI and WHR in female athletes. Cho et al. (2006) indicated that RBP4 levels were not associated with body mass in obese Korean women (8). Mansouri et al. (2011) also demonstrated that RBP4 levels were not associated with BMI in young male students (9). However previous study demonstrated that RBP4 concentration was higher in

middle-aged women with higher BMI than young women (10). These discrepant results may be attributed to differences in subject populations because our subjects were athletes while non-athlete middle-aged women were participated in the Lim et al. study. The results showed that body fat percentage did not significant change after 8 weeks exercise, thus it seems that the lack of effect of exercise training on RBP4 in the present study might be due to the absence of reductions in body fat percentage. Studies demonstrated that the response of RBP4 to 1 month of exercise training was variable (6), and RBP4 levels were decreased after exercise mainly in the subjects having higher RBP4 levels at baseline (10). Research result showed that the basic level of RBP4 appeared to be the only predictor of after exercise RBP4 concentration (9). Our results demonstrated that RBP4 levels at baseline were lower than the subjects that participated in the Lim et al. study, suggesting that athletes might have lower levels of RPB4 than non-athlete.

On the other hand, elevated RBP4 levels have been reported in subjects with insulin resistance and type 2 diabetes (6,9) whereas other studies showed no relationship between circulating RBP4, obesity, and insulin resistance (9,1,18). Our results showed that there was no significant relationship between RBP4 levels and insulin resistance determined by HOMA-IR. Cho et al. (2006) and Mansouri et al. (2011) also indicated that RBP4 levels were not associated with insulin resistance (8,9) while, Lim et al. (2008) showed that there was a positive relationship between RBP4 concentration and insulin resistance (10). In our study, there was virtually no change in fasting glucose and insulin and HOMA-IR after 8 weeks training, however Lim et al. (2008) reported that insulin resistance decreased after 10 weeks exercise training in young and middle-aged women (10). We had some limitations in this study. We did not measure inflammatory markers and cardiovascular risk factors. Previous studies demonstrated that RBP4 concentrations were associated with inflammatory markers such as CRP (19) and cardiovascular risk factors such as triglyceride (11), total cholesterol (8) and LDL cholesterol (8). If we could measure these markers, we could carefully explain the changes of RBP4 concentrations in response to 8 weeks exercise training in female athletes.

5. Conclusion

Our results showed that serum RBP4 levels were not affected by exercise intensity in female athletes. Additional research is needed in this field.

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