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Impact of resistance training on hepcidin levels and iron status in overweight/obese girls with and without iron stores deficient

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Abstract

Background: Increasing fat mass by increasing inflammation can be responsible for causing anemia. Exercise can reduced hepcidin and improves the iron status, but the type and intensity of exercises are very important. The aim of this study was to compare two different intensities of resistance training on hepcidin levels, iron status and body composition in obese/overweight girls.

Method: 40 university students (18-22years old) with 35>BMI≥25 voluntarily participated in the study. Participants were divided into two groups of20, by blood sampling and based on serum ferritin (>30ng/ml or ≤30ng/ml). Subjects in each group were randomly assigned to one of the moderate or high-intensity training groups. Resistance training was performed 8 weeks, four days a week, and each session for one hour, with an elastic-band. The levels of iron, hepcidin, TIBC, ferritin, hemoglobin, before and after intervention were collected with the blood samples. T-test and ANCOVA were used to compare the groups.

Results: T-test showed, decrease in body composition factors, Iron status, hepcidin, and inflammatory markers. The result of ANCOVA showed a significant difference between two intensity of training in groups with serum ferritin>30ng/ml, in hepcidin. (P<0.05).

Conclusion: resistance training with two different intensities can reduce hepcidin, ferritin and BMI in obese girls with and without iron deficiency and improve body composition. Baseline ferritin level seems to be effective on hepcidin levels after intervention. Keywords: resistance training, hepcidin, iron status, obesity.

Keywords: Resistance training, overweight/obesity, inflammation, iron status

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1. Introduction

Obesity and iron deficiency are the two serious problems in today's world (1). The association between obesity and iron deficiency has been confirmed in adults as well as in children and adolescents (2,3). Obesity can reduce the absorption of iron supplementation for treatment (4). The Possible mechanisms to explain the association between obesity and anemia include decreased iron absorption from food, increased iron requirement due to increased body mass, and increased blood volume. Of course, in women, menstruation and fat tissue inflammation can increase iron deficiency. Another possibility for this connection is mild inflammation following increased fat mass. After surgery, the inflammation has decreased and the iron status has improved (1). Reducing inflammation by reducing the hepcidin hormone can improve the iron status and vice versa, by increasing its concentration, iron absorption decreases. Hepcidin is a peptide hormone that is mainly made by the liver and as a regulator of iron, binding to Ferroportin-1, regulates the plasma iron by absorbing iron from the intestine, releasing iron from liver, restoring iron through macrophages (5). Researchers believe weight loss can significantly decrease hepcidin concentrations, thereby increasing iron intestinal absorption and improving the iron status of these people (6). A very low-calorie diet will further reduce the obesity of children's iron stores and calorie restriction programs may reduce iron intake in obese people, especially in women. Treating iron deficiency by getting more from diet or supplements, due to the presence of inflammation in people and reduced iron absorption, cannot be a suitable solution to improve iron status (7).

However physical activity can reduce the size of fat cells(8). and the weight loss program in obese children is associated with a decrease in BMI(6), reduced hepcidin levels and improved iron status in obese children(6,9). However high-intensity exercises have shown better results on reducing inflammation than moderate-intensity training (10), the long times training and increased severity can increase the expression of hepcidin and lead to anemia (11).

4 weeks of high-intensity interval aerobic exercise in women has been shown to reduce iron and hepcidin levels, however the hepcidin expression decreases after moderate-intensity training)11(. Resistance Training (RT) can reduce TNF-α expression and is an effective way to prevent and delay inflammation (12). The intense RT, effectively reduced iron deficiency in mice (13) and moderate-intensity RT in iron-deficient young women, improved iron status

(13). In addition to the type of exercise, hepcidin levels change after physical activity are different based on the initial state of iron (14). Therefore, the effect of RT on the reduction of inflammation and on improving the status of iron and hepcidin, because of the iron status in the base, seems necessary.

Materials and methods:

2.1.Subjects:

This quasi-experimental study is a quadruple with pretest-posttest measurements. For this purpose, female students of Isfahan University with ages (18-22) who were willing to participate in the project were subjected to BMI measurement, and those with a BMI above 25 were selected and familiarized with the process of the project. And after completing the consent form, they performed the first stage of blood sampling at the end of the follicular phase. Subjects were divided into two groups based on iron status (<30 ng/ml or ≤30 ng/ml), Then, the subjects of each group were randomly assigned to two subgroups for high or moderate-intensity training. In the first group, individuals with ferritin≤30, moderate-exercise, and the second group with ferritin≤30 exercises of high-intensity. Individuals with ferritin>30 were assigned to either the third group with moderate-intensity of training or the high-intensity group. Exclusion criteria include the participation in sport exercises, the use of a specific diet or the use of iron supplements, and any other drug for a particular disease in the last 6 months, pregnancy and absorptional problems.

Practice protocol

In this research, elastic-bands were used for resistance exercises.Elastic-bands with its rubber resistance has different characteristics than free weights, including that it does not rely on gravity to produce elastic bands strength. Therefore, various patterns of speed and movement can be implemented with this device. The use of this device makes it easier to carry out exercises because of its low cost and availability, ease of use, noise, and safety. Subjects underwent RT for 10weeks, in a way that during the first two weeks, the subjects became familiar with this type of exercise, and after having determined the 15RM, they performed RT with cache for 8weeks. The first color chosen to start exercises was blue because the subjects were able to practice between 15and20 repetitions.

The frequency of maximal repeat after 4weeks was reevaluated and increased by more than 30repetitions. To determine the severity for the subjects, a different elasticity in the range of permissible joint motion was used, in a way that the difference in elasticity between the two moderate and intense training groups was25%, based on the elastic-repetition number(15). The resistance between the two groups was different. In the first 4 weeks, two groups did moderate intensity exercises with 75% elasticity and two groups did intense exercises with 100% elasticity. The resistance between the two groups was different. In the 4weeks, the amount of elasticity increased by25% for all four groups.Each exercise with three sets and the number of 12repetition were fixed for all groups. The amount of rest between each set in all groups was 90seconds and between each move was 30seconds. The exercises were included hip abduction and adduction, flexion, and extension of the hip, parotid, upper trunk, crown, shoulder abduction, elbow flexion and, elbow extension(15). A blood sample was taken from the vein to measure hepcidin, ferritin, iron, TIBC, then the serum and plasma were separated from each other and stored at -30°C. According to the suggestion of Peling et al. (2009) ,The best time to measure hepcidin is 24 hours after the last session of the exercise, which can be measured and investigated the effects of other factors, such as iron (16).

Body composition

Height with a precision of 1cm, bodyweight with an accuracy of 0.1kg, BMI was calculated by dividing body weight into height squared. If the BMI is between 25-29.9kg/m2, it will be in the overweight range, and if it is more than 30kg/m2, it will be in the range of obesity (17).

Iron status

TIBC concentration was measured by the RID method. A photometric method was used with Ferro Zine to measure serum iron and ferritin levels were measured with the vanguard kit. This kit was based on sandwich method and using monoclonal antibodies. Iron deficiency was estimated with serum ferritin concentration ≥ 30. The choice of ferritin level of 30 ng/mL is based on the iron deficiency determination standard of the Royal Australian Pathology College. Circulating levels of ferritin between 30-50ng/L, based on the recommendation of Garviken et al ,is declared to be the sub-optimal levels and between (50-100ng/L) is the optimum level (18).

Hepcidin

The amount of hepcidin was measured using the relevant kit from ESTABIOPHARM and number (Cat.no: CK-E90185) by ELIZA method.

The average and standard deviation of the data are recorded in Table No. 1. The normality of the data was checked using the Shapiro-Wilk test. A two-way analysis of variance was used to compare between groups, so that the effect of ferritin levels, resistance training and the mutual effect of training and ferritin levels on the variables were tested. Correlated t-test was used to check the change of variables compared to the baseline. $P<0.05$ was also considered as a significant level.

Results:

Participants' profile and baseline measurements

The results showed no significant interactions between ferritin levels and exercise intensity (All $P>0.05$). There was no significant difference between the intense and moderate mode of exercise($P>0.05$). However, there was a significant difference between low and high levels of Ferritin for Hepcidin($P=0.017$), but other variables showed no significant differences (All $P>0.05$)(Table 1).

Changes from baseline

Ferritin ≤30- Intense-exercise

Significant decreases were observed in BMI, Hepcidin, and Ferritin compared to the baseline measurements(All $P<0.05$).

Ferritin ≤30- Moderate-exercise

Significant falls were observed in weight, BMI and Hepcidin compared to the baseline measurements(All $P<0.05$).

Ferritin >30- Intense-exercise

Significant reductions were observed in BMI, Hepcidin, and Ferritin compared to the baseline measurements(All $P<0.05$).

Ferritin >0- Moderate-exercise

Significant decreases were observed in weight, BMI, hepcidin, and Ferritin as compared to the baseline measurements(All $P<0.05$)(Table 2).

Effect of exercise and ferritin levels

The results showed no significant interactions between ferritin levels and exercised intensity for the main outcomes after adjusting for age and baseline measurements (All $P>0.05$). However, a significant difference was observed between the intense and moderate mode of exercise in TNF-α($P<0.05$), but there were no significant differences between the mode of exercise for other main variables(All $P>0.05$). However, there was a significant difference between low and high levels of Ferritin for Hepcidin($P=0.002$), but other variables showed no significant differences(All $P>0.05$) (Table 3, Figure 1).

Table 1: Participants' profile and baseline measurements of anthropometric indices and biochemistry outcomes

Variables	**ferritin≤30**				**ferritin>30**				**Interaction P-value**	**Ferritin Effect P-value**	**exercise P-value**
	Intense-exercise (n=10)		**Moderate-exercise (n=10)**		**Intense-exercise (n=10)**		**Moderate-exercise (n=10)**				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Age(year)	20.20	1.23	21.00	1.33	21.10	1.10	20.40	.84	.055	.680	.891
Weight(kg)	73.35	11.36	73.85	7.52	76.95	15.15	75.25	11.13	.766	.500	.871
BMI(kg/m^2)	28.49	4.19	28.78	2.59	29.13	5.13	29.47	3.89	.985	.609	.806
Iron(mcg/d)	89.80	20.58	78.90	22.59	94.21	27.21	92.90	24.83	.530	.232	.425
TIBC(mcg/d)	384.80	26.13	399.60	14.46	382.40	21.12	392.10	24.60	.717	.482	.087
Hem(g/d)	13.06	1.26	13.41	2.64	13.81	.72	13.47	.72	.486	.414	.992
Hepcidin(Pg/m)	1457.9	608.5	1364.1	537.7	1097.7	323.3	1016.2	187.4	.966	**.017**	.539
Ferritin(ng/ml)	22.05	7.92	22.90	3.70	52.22	16.16	56.60	19.99	.683	**<.001**	.546

Table 2: Changes from baseline of anthropometric indices and biochemistry outcomes

Variables	**ferritin≤3٠**						**ferritin>30**					
	Intense-exercise (n=10)			**Moderate-exercise (n=10)**			**Intense-exercise (n=10)**			**Moderate-exercise (n=10)**		
	MD	**95%CI**	**P-value**	**MD**	**95%CI**	**P-value**	**MD**	**95%CI**	**P-value**	**MD**	**95%CI**	**P-value**
Weight(kg)	-1.40	-2.92, .12	0.067	-2.95	-3.93, -1.97	**<0.001**	-1.60	-3.39, .19	0.074	-2.55	-3.90, -1.20	**0.002**
BMI(kg/m^2)	-1.16	-2.07, -.26	**0.018**	-2.05	-2.98, -1.12	**0.001**	-1.45	-2.16, -.74	**0.001**	-2.24	-4.46, -.02	**0.048**
Iron(mcg/d)	-10.40	-32.24, 11.44	0.310	-0.70	-15.65, 14.25	0.918	-10.31	-35.07, 14.44	0.371	-5.20	-22.15, 11.75	0.505
TIBC(mcg/d)	-10.30	-35.13, 14.53	0.373	-5.70	-16.06, 4.66	0.245	-1.60	-32.16, 28.96	0.908	3.20	-20.50, 26.90	0.767
Hem(g/d)	0.46	-0.54, 1.46	0.325	-0.64	-2.24, .96	0.388	0.56	-1.66, 2.78	0.583	0.10	-0.35, .55	0.626
Hepcidin(Pg/m)	-429.1	-723.0, 135.3	**0.009**	-394.7	-598.5, -191.0	**0.002**	-197.3	-288.2, 104.5	**0.001**	-66.6	-127.3, -5.9	**0.035**
Ferritin(ng/ml)	-11.68	-17.33, -6.03	**0.001**	-5.72	-13.93, 2.49	0.149	-24.39	-35.79, -12.99	**0.001**	-17.00	-32.35, -1.65	**0.034**

Table 3: Effect of intervention on anthropometric indices and biochemistry outcomes in low and high levels of ferritin

Variables	Interaction P-value	Ferritin Effect P-value	Exercise P-value
Weight(kg)	.947	.789	.057
BMI(kg/m^2)	.508	.791	.156
Iron(mcg/d)	.562	.860	.394
TIBC(mcg/d)	.438	.469	.674
Hem(g/d)	.857	.489	.249
Hepcidin(Pg/m)	.345	**.002**	.335
Ferritin(ng/ml)	.973	**.019**	.171

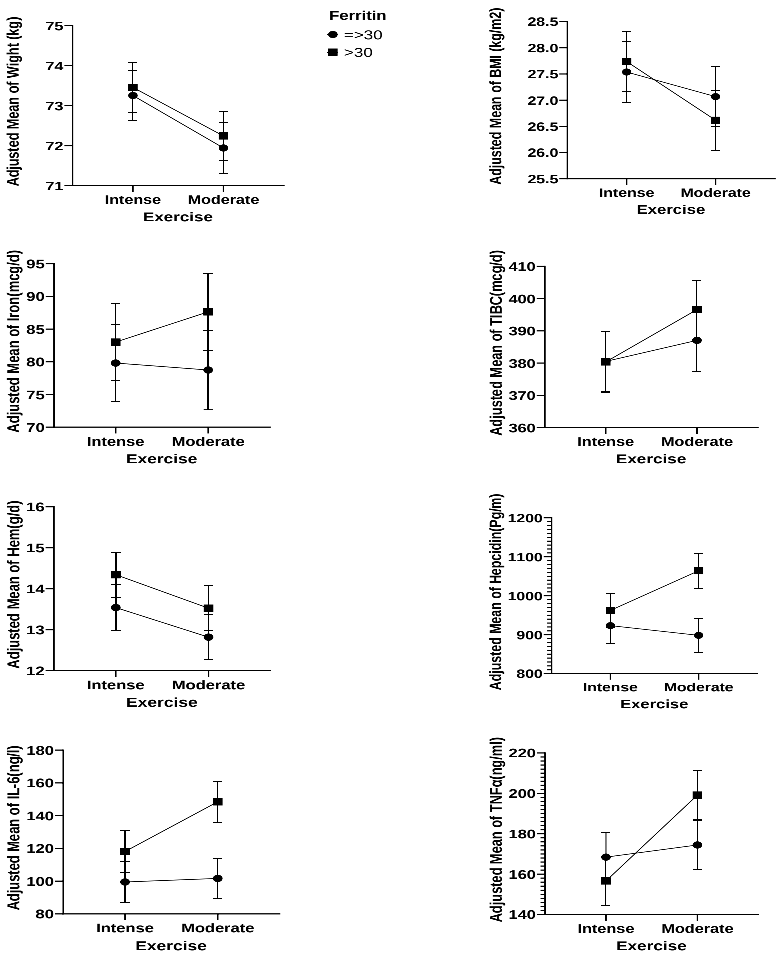

Figure 1: Effect of exercise and ferritin levels on anthropometric indices and biochemistry outcomes

Discussion:

According to the results of this study, Based on the results of the present research, resistance training decreased body composition factors, iron status, and hepcidin in all four training groups. Weight, BMI and BF decreased in all four groups, but no significant difference was observed between the two training intensities. However, only baseline levels of ferritin (<30 ng/ml or ≤30 ng/ ml) caused a significant difference in hepcidin levels.

In the study of calle et al. and Delshad et al. was observed a decrease in body weight and body fat mass after 12 weeks of resistance training with banding (19,20) as well as fat percentage and BMI (20), which with The results of the present study that reduced weight and BMI are consistent. However, in the study of Delshad et al. (2011) comparing high and medium training intensities, high-intensity training caused weight loss and BMI, but in the current study, the effect of training intensity did not create a significant difference between the groups (19). The reason for the difference in the results of the studies may be due to the different length of the training period.

In the present study, serum ferritin, TIBC, and serum iron decreased compared to before exercise, only in people with ferritin>30, a significant decrease in ferritin was observed, and it was insignificant in the rest of the cases. In the study of Matsu et al (2000), young women with iron deficiency performed moderate-intensity resistance training and reported improvements in their iron status (21), but McClung et al(2009) reported a decrease in iron status indices following 8 weeks of military training and attributed it to a lack of dietary iron intake, while dietary iron absorption is also affected by exercise training (22). The reason for the difference in the results of these two studies was the intensity of the exercises, so that moderate intensity exercise improved iron status. in a study on an animal model done by Fuji et al (2014), reported that iron absorption during 6 weeks of exercise in the intervention group was lower than in the no-exercise group, while there was no difference in the amount of total body iron sources between the two control and experimental groups. Fuji explained the reason is the use of iron from the metabolism of erythrocytes in macrophages (23). About 70% of the body's iron is used to make hemoglobin (24) Exercise increases the synthesis of bone marrow, and by increasing the use of iron metabolism, such as the destruction of red blood cells, it can improve the iron status (23). In the present study, the iron content of muscles and organs has not been investigated. However, iron deficiency is probably due to the new iron distribution in the body and erythropoiesis. Besides, exercise duration may not be sufficient to produce significant differences in hemoglobin and iron concentrations in the present study.

In the study of Ryan et al. (2021), the amount of iron reserves of the whole body in the liver and skeletal muscle was checked and reported a decrease in iron reserves following the moderate and high intensity exercise, and as a result, the modification in the exercise training was confirmed in iron reserves in adults (25). As mentioned, serum ferritin, as an acute phase inflammatory factor, is higher in obese children and increases with increasing BMI, so a decrease in ferritin can be a sign of decreased inflammation following a decrease in BMI (5). On the other hand, the reduction of ferritin in the group with ferritin reserves ≤30 may be due to a protective mechanism to absorb more iron and increase iron serum, because by reducing the amount of iron, the synthesis of hepatic hepcidin decreases and thus Ferrsportin channels open to absorb iron from the intestine and transfer it to the bloodstream (5). Several studies have investigated the effect of initial iron status on post-exercise hepcidin levels and have reached different results. In the study of Arosperger et al (2013), regardless of the initial iron status, hepcidin concentration decreased after aerobic exercise, but no significant difference was observed in hepcidin after exercise according to the normal iron status or lack of iron stores (26). According to Borion et al.'s study (2001), ferritin values<30 caused a difference in the response of hepcidin to exercise and dietary iron (27). The results of Peelling et al(2014) showed that the amount of hepcidin does not change after exercise in the subjects who have ferritin<30, but it increases significantly in those who have ferritin>30 (18). Therefore, hepcidin changes after exercise may be dependent on baseline values. Also, in the study of Karl et al(2010), the amount of basal iron was effective on hepcidin concentration after exercise(28). Cortaz et al. (2015) also reported a positive correlation between blood ferritin and hepcidin concentration (29). In the study of Amato et al(2010), the weight loss of obese children was associated with a decrease in hepcidin serum,and a significant improvement in iron absorption was observed (6). Indeed, it was found that decreasing BMI decreased circulating levels of hepcidin and increased iron absorption. In the present study, the reduction of hepcidin was more in the group with low ferritin reserves, which confirms the results of these studies. Many studies have shown that hepcidin levels increase after exercise. The intensity of exercise is one of the factors that can affect the amount of hepcidin after exercise. In Liu et al.'s (2011) study, 5 weeks of intense treadmill running training in mices showed that the level of liver hepcidin expression increased and caused anemia. This result has also been observed in human samples (30). Also, an increase in hepcidin mRNA expression with a decrease in serum iron and ferritin was observed after 5 weeks of training in mices.Therefore, intense exercise increases the amount of hepcidin and decreases the amount of iron absorption (30). However, moderate-intensity resistance training improved iron status, so that hepcidin expression decreased after moderate-intensity training (13). Also, in Liu et al.'s (2006) study, increased expression of DMT-1 and FPN-1 in intestinal cells led to increased iron absorption following moderate intensity exercise compared to the control group (31). Erythropoietic activity and anemia can reduce hepcidin synthesis by activating the BMP pathway and ultimately lead to increased iron absorption from the intestine, consume iron of macrophages and liver under iron deficiency conditions and increased iron requirement (11,32). Therefore, it seems that the reason for the decrease in hepcidin levels in this study is the homeostatic regulation of iron in the body due to the increased need for iron.

Conclusion:

According to the results of the this study, it is possible that performing resistance exercises with elastic bands for 10 weeks and with different intensity does not improve the iron status variables, but the basic iron status can be effective on the iron and hepcidin status after the exercises. Therefore, it is important to pay attention to the amount of iron reserves in order to lose weight.

There is not Conflicts of interest.

Ethical Considerations: Code of ethics with the number 1396.038IR.UI.REC. Confirmed.

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