Impact of resistance training and basic ferritin on hepcidin, iron status and some inflammatory markers in overweight/obese girls

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Background: Exercise can reduce hepcidin, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 and improve the iron status, but the intensity of exercises is very important. This study will compare the effect of resistance training (RT) intensity on hepcidin levels, iron status, and inflammatory markers in overweight/obese girls with and without iron stores deficient. **Materials and Methods:** In this quasi-experimental study, 40 students of the University of Isfahan (18–22-year old, with 35 > body mass index [BMI] ≥25) voluntarily participated in the study. Participants were divided into two groups with 20 participants, based on serum ferritin (>30 ng/ml or ≤30 ng/ml). Participants in each group were randomly and equally assigned to one of the moderate or high-intensity training groups. RT was performed 8 weeks, 4 days a week, and each session for 1 h, with an elastic band. The iron levels, hepcidin, total iron-binding capacity, ferritin, hemoglobin, TNF-α, and IL-6 before and after intervention were collected with the blood samples. Two-way analysis of variance was used to assess the impact of exercise and ferritin level and their interaction, and the paired test was utilized for test changes from baseline. **Results:** There are no significant interactions between ferritin levels and exercise intensity for the main outcomes (all $P > 0.05$). The significant impact of the mode of exercise was observed in TNF-α ($P < 0.05$), and a significant difference between low and high levels of ferritin was observed in hepcidin ($P = 0.002$). Besides, in all four groups, significant decreases were observed in BMI (28.00 ± 3.00 to 27.00 ± 3.00), hepcidin (1234.02 ± 467.00 to 962.06 ± 254.00), and TNF-α (223.00 ± 99.00 to 174.00 ± 77.00) compared to the baseline measurements (all $P < 0.05$). **Conclusion:** Basal ferritin levels appear to be effective on hepcidin levels, TNF-α, and IL-6 after the intervention. RT with two different intense can reduce BMI, hepcidin, ferritin, and TNF-α in all groups. It seems that performing RT reduces inflammation and hepcidin in obese/overweight participants with different iron stores.

Key words: inflammation, iron status, overweight/obesity, resistance training

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INTRODUCTION

Obesity and iron deficiency are the two main problems in today's world.[1] The association between obesity and iron deficiency has been confirmed in adults, children, and adolescents.[2,3] Obesity can reduce the absorption of iron supplementation for treatment.[4]

Increasing fat cells causes hypoxia and penetration of the production of local cytokines, so it increases the expression of interleukin-6 (IL-6) and the tumor necrosis factor-α (TNF-α). Increased expression of hepcidin with increased circulating inflammatory cytokines such as IL-6 and TNF-α has been observed.[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 mainly made by the liver. As a regulator of iron, binding to ferroportin-1 regulates the plasma iron by absorbing iron from the intestine, releasing iron from the liver, restoring iron through macrophages.[5] Weight loss can significantly decrease hepcidin concentrations, increasing intestinal iron absorption, and improving these people's iron status.[6]

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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 women. After surgery (abdominal fat suction), inflammation has decreased, and iron status has improved, but it cannot be a good way 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 body mass index (BMI),[6] reduced hepcidin levels, and improved iron status in obese children.[6,9]

However, high-intensity exercises have shown better results in 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]

Four weeks of high-intensity interval aerobic exercise in women has been shown to reduce iron and hepcidin levels. However, hepcidin expression decreases after moderate-intensity training.[11] Resistance training (RT) can reduce TNF-α expression and effectively 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 exercise, hepcidin levels change after physical activity are different based on iron's initial state.[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

Participants

In this quasi-experimental study with pretest-posttest measurements, 40 female students of the University of Isfahan participated in this study, during March 21, 2017–June 6, 2017. Inclusion criteria were willing to participate in the study, age of 18–22 years and BMI levels of 25–35. Those in other sports exercises, use a specific diet or use iron supplements, any other drug for a particular disease in the past 6 months, pregnancy, and absorption problems were excluded from the study.

Study size

The study's sample size was computed using the information obtained from a pilot on five participants on hepcidin as the main variable. Considering 80% power, 95% confidence, and a two-tailed test, we estimated a sample size of 18 people per group, which we increased to 20 to account for 10% attrition.

Randomization

After completing the consent form, the participants completed the first stage of blood collection at the end of the follicular phase and were divided into two groups with ferritin (>30 ng/ml or ≤ 30 ng/ml). Then, each group's participants were randomly and equally assigned to two subgroups for high or moderate-intensity training, based on a randomized block procedure of size 2 for intense/moderate groups considering the ferritin level as strata. Exit criteria include participation.

Exercise protocol

In this research, elastic bands were used to conduct RT. Elastic bands with its rubber resistance have 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. This device's use makes it easier to carry out exercises because of its low cost and availability, ease of use, noise, and safety. Participants underwent RT for 10 weeks, in a way that during the first 2 weeks, the participants became familiar with this type of exercise, and after having determined the 15 RM, they performed RT with cache for 8 weeks. The first color chosen to start the exercises was blue because the participants could practice between 15 and 20 repetitions. The frequency of maximal repeat after 4 weeks was reevaluated and increased by more than 30 repetitions. A different elasticity in the range of permissible joint motion was used to determine the participants' severity. The difference in elasticity between the two moderate and intense training groups was 25%, based on the elastic-repetition number.[15] The resistance between the two groups was different. In the 4 weeks, the amount of elasticity increased by 25% for all four groups.[15] Each exercise with three sets and the number of 12 repetition were fixed for all groups. The amount of rest between each set in all groups was 90 s and between each move was 30 s. The exercises included abduction and hip adduction, flexion, and extension of the hip, parotid, upper trunk, crown, shoulder abduction, elbow flexion, and elbow extension.[15]

Main variables

A blood sample was taken from the vein to measure hepcidin, ferritin, hemoglobin, iron, total iron-binding capacity (TIBC), TNF-α, and IL-6. Then, the serum and plasma were separated from each other and stored at −30°C. The best time to measure hepcidin is 24 h after the last session of the exercise measured and investigated the effects of other factors, such as iron.[16]

Measurements

Body composition

Height with a precision of 1 cm, bodyweight with an accuracy of 0.1 kg, BMI was calculated by dividing body weight into height squared. If the BMI is between 25 and

29.9 kg/m², it will be in the overweight range, and if it is more than 30 kg/m², it will be in the range of obesity.[17]

Iron status

TIBC concentration was measured by the radio immunodiffusion method. A photometric method was used with ferrozine to measure serum iron, and ferritin levels were measured with the vanguard kit. Ferritin 30 ng/ml choice is based on the Iron Deficiency Iron Curriculum of the Australian Royal Pathology Faculty. Circulating ferritin levels between 30 and 50 ng/L are declared to be the suboptimal levels and between 50 and 100 ng/L are the optimum level.[18] The levels of hepcidin, IL-6, and TNF-α were measured using the corresponding kit from Estabiopharm Company, with the numbers (Cat. no: CK-E90185), (Cat. no: CK-E306436), and (Cat. no: CK-E30635) were measured by the ELIZA method.

Ethical considerations

The institutional review board of Isfahan University approved the protocol of this study. The participants gave the written informed consent and were free to exclude the study (Ethics code: IR.UI.REC.1396.038, Clinical trial code: IRCT20200406046974N1).

Statistical analyses

Data were expressed as mean and standard deviation in tables and mean and standard errors in figures. The normality of data was assessed by the Shapiro–Wilk test and reviewed by the distribution measures (skewness within ± 1.5 and kurtosis within ± 2), and the normality of variables was confirmed. To compare the participants' profile and baseline measurements, considering the ferritin levels and the exercise and possible interactions, two-way analysis of variance has used. In addition, we utilized this analysis to assess the impact of the ferritin levels and the exercise and their possible interaction on the main variables after adjusting for baseline measures and participants' age. Paired t-tests were used to test each main variable's baseline changes in each ferritin levels and the exercise groups. IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA) was used to analyze data at a 0.05 significance level. GraphPad 8.1 was utilized to draw the figures. There was no missing data in the study, and analyses were carried out with an intention to treat approach.

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 falls were observed in weight, BMI, hepcidin, IL-6, and TNF-α compared to the baseline measurements (all $P < 0.05$)
- Ferritin ≤30 – moderate-exercise: significant decreases were observed in BMI, hepcidin, TNF-α, and ferritin compared to the baseline measurements (all $P < 0.05$)
- Ferritin >30 – intense exercise: significant decreases were observed in weight, BMI, hepcidin, TNF-α, and ferritin as compared to the baseline measurements (all $P < 0.05$) [Table 2]
- Ferritin > 30 – moderate exercise: significant reductions were observed in BMI, hepcidin, TNF-α, and ferritin compared to the baseline measurements (all $P < 0.05$).

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 and Figure 1].

DISCUSSION

According to this study results, performing RT with two different intensities significantly impacted hepcidin and TNF-α. However, there were no significant differences in serum iron, hemoglobin, TIBC, weight, and BMI between 2 training groups in overweight/obese girls.

RT can convert fat mass to muscle mass and maintain high muscle mass and reduce risk factors for obesity. Adipose tissue is reduced after exercise without changing body weight.[12] However, 12 weeks of RT with banding significantly reduced weight, especially body fat.[19] Elastic band training performed by overweight/obese women, with 80%, 85%, and 100% of 10-RM for 12 weeks, significantly reduced body fat percentage, fat, and subcutaneous fat.[20] There was a significant decrease in BMI in all four groups and body weight in two moderate groups compared to baseline in the present study. The main reason might be insufficient exercise duration.

There is a direct relationship between muscle mass and cytokine levels.[12] Therefore, the exercise's anti-inflammatory effects depend on the type of exercise and are independent

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

Variables	Ferritin ≤30, mean±SD (*n*=10)		Ferritin >30, mean±SD (*n*=10)		*P*		
	Moderate exercise	Intense exercise	Moderate exercise	Intense exercise	Interaction	Ferritin effect	Exercise
Age (years)	20.20±1.23	21.00±1.33	21.10±1.10	20.40±0.84	0.055	0.680	0.891
Weight (kg)	73.35±11.36	73.85±7.52	76.95±15.15	75.25±11.13	0.766	0.500	0.871
BMI (kg/m²)	28.49±4.19	28.78±2.59	29.13±5.13	29.47±3.89	0.985	0.609	0.806
Iron (mcg/day)	89.80±20.58	78.90±22.59	94.21±27.21	92.90±24.83	0.530	0.232	0.425
TIBC (mcg/day)	384.80±26.13	399.60±14.46	382.40±21.12	392.10±24.60	0.717	0.482	0.087
Hemoglobin (g/day)	13.06±1.26	13.41±2.64	13.81± 0.72	13.47±0.72	0.486	0.414	0.992
Hepcidin (Pg/m)	1457.9±608.5	1364.1±537.7	1097.7±323.3	1016.2±187.4	0.966	0.017	0.539
IL-6 (ng/l)	150.33±60.65	152.30±51.07	124.45±49.08	165.01±54.70	0.266	0.702	0.222
TNF-α (ng/ml)	213.46±114.11	239.45±71.93	252.59±123.75	189.28±33.36	0.138	0.852	0.530
Ferritin (ng/ml)	22.05±7.92	22.90±3.70	52.22±16.16	56.60±19.99	0.683	<0.001	0.546

SD=Standard deviation; BMI=Body mass index; TIBC=Total iron-binding capacity; IL-6=Interleukin-6; TNF-α=Tumor necrosis factor-alpha

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

Variables	Ferritin ≤30 (*n*=10)						Ferritin >30 (*n*=10)					
	Moderate-exercise			Intense-exercise			Moderate-exercise			Intense-exercise		
	MD	95% CI	*P*	MD	95% CI	*P*	MD	95% CI	*P*	MD	95% CI	*P*
Weight (kg)	−1.40	−2.92-0.12	0.067	−2.95	−3.93-−1.97	<0.001	−1.60	−3.39-0.19	0.074	−2.55	−3.90-−1.20	0.002
BMI (kg/m²)	−1.16	−2.07-−0.26	0.018	−2.05	−2.98-−1.12	0.001	−1.45	−2.16-−0.74	0.001	−2.24	−4.46-−0.02	0.048
Iron (mcg/day)	−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/day)	−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
Hemoglobin (g/day)	0.46	−0.54-1.46	0.325	−0.64	−2.24-0.96	0.388	0.56	−1.66-2.78	0.583	0.10	−0.35-0.55	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
IL-6 (ng/l)	−45.53	−102.33-11.27	0.103	−53.13	−83.87-−22.39	0.004	−15.17	−48.62-18.28	0.332	−10.53	−53.13-32.07	0.590
TNF-α (ng/ml)	−49.83	−67.66-−32.00	<0.001	−55.07	−101.97-8.17	0.026	−76.64	−102.26-−51.02	<0.001	−14.59	−39.50-10.32	0.218

CI=Confidence interval; MD: Mean difference; BMI=Body mass index; TIBC=Total iron-binding capacity; IL-6=Interleukin-6; TNF-α=Tumor necrosis factor-alpha

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

Variables	*P*		
	Interaction	Ferritin effect	Exercise
Weight (kg)	0.947	0.789	0.057
BMI (kg/m²)	0.508	0.791	0.156
Iron (mcg/day)	0.562	0.860	0.394
TIBC (mcg/day)	0.438	0.469	0.674
Hemoglobin (g/day)	0.857	0.489	0.249
Hepcidin (Pg/m)	0.345	0.002	0.335
IL-6 (ng/l)	0.945	0.059	0.958
TNF-α (ng/ml)	0.055	0.587	0.044

BMI=Body mass index; TIBC=Total iron-binding capacity; IL-6=Interleukin-6; TNF-α=Tumor necrosis factor-alpha

of weight loss. A short course of RT can produce long-term anti-inflammatory effects, positively regulating some cytokines.[12] RT also harms the production of TNF-α by increasing the production of IL-6 from contracting muscle, which can also be effective in reducing both local inflammations of the muscle and systemic inflammation.[12] In fact, through increasing lipolysis, these cytokines can increase lipid oxidation through AMPK.[19]

The TNF-α levels reduced after an RT period with the intensity of 70% to 80% 1RM[21] and moderate to intense RT,[22] while a moderate exercise period did not show a change in the expression of TNF-α. Despite a decrease in total body fat following RT and reducing visceral fat, no significant changes in IL-6 levels were observed with aerobic training,[23] which consistent with the results of this study. Twelve weeks of moderate to severe combination training in overweight/obese and overweight children do not affect levels of IL-6 and TNF-α.[24] However, in a 1-year weight loss program in overweight/obese children, IL-6 levels were improved at the follow-up stage.[9] Overall, it seems that changing IL-6 circulation levels will require a longer training period, while TNF-α is affected by exercises in a shorter period and responds to the exercises. In this study, moderate RT resulted in significant reductions in TNF-α levels, but the IL-6 levels were not affected except ferritin ≤ 30 and moderate exercise mode.

Reducing inflammation can reduce baseline hepcidin levels. Five weeks of intense treadmill training in mice increases hepcidin expression and induced anemia due to exercise[25] and reduction of serum iron and ferritin.[25,26] This result has also been observed in human participants.[11] However, increasing expression of divalent metal transporter-1 and ferroportin-1 (FPN-1) in intestinal cells resulted in an increase in iron absorption following a moderate-intensity training compared to the control group.[27] Moderate

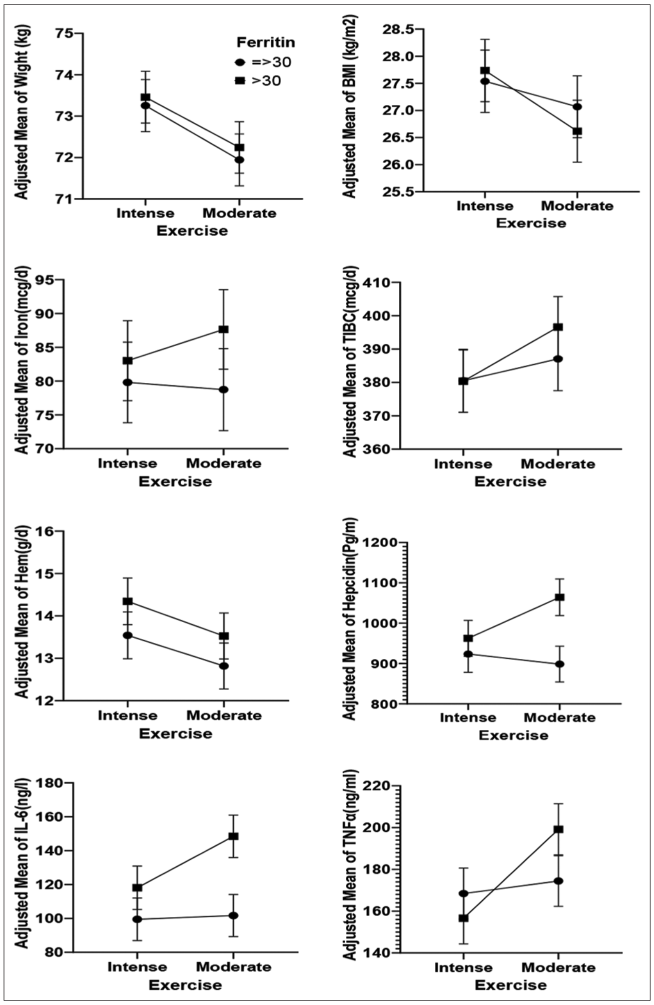

Figure 1: Effect of exercise and ferritin levels on anthropometric indices and biochemistry outcomes

intensity RT also improves the iron status, as the amount of hepcidin expression decreases after moderate-intensity training.[13]

Another factor is the effect of the initial iron status on hepcidin levels after training,[15] which has led to different results. However, several studies confirm the effect of the initial iron status on hepcidin levels after training.[11,28,29] Erythropoietic activity and anemia can reduce hepcidin synthesis and ultimately lead to increased iron absorption from the intestine, iron macrophages, liver, and iron deficiency in plasma increase the need for the body. Other studies also expressed an increase in the level of erythropoietin due to the reduction of hepcidin.[11] Therefore, the reduction of hepcidin levels in the present study was the regulation of iron in the body due to the increased need for iron. There was also a further reduction in the hepcidin group with ferritin < 30.

About 70% of the body's iron is used to make hemoglobin.[30] 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.[31] 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.

As an acute inflammatory factor, serum ferritin is more common in overweight/obese children and increases BMI. Therefore, reducing ferritin can be a sign of reducing inflammation, following a reduction in BMI. On the other hand, reducing ferritin in a group with ferritin ≤ 30 may be due to a protective mechanism for absorbing more iron in them and increasing iron in the serum. As the amount of iron decreases, liver hepcidin synthesis decreases, and through this, the FPN-1 can open the intestines and transfer them to the bloodstream.[5]

The results of the study should be interpreted in the shadow of some limitations. First, the study's sample size was small, and some nonsignificant results may be due to the statistical tests' small power. Second, the study population consisted of university students with a limited age group, which restrict the generalizability of the results. A study in various populations with different age groups is recommended to cover this issue. Finally, insufficient exercise duration might cause some weak impact, especially weight and BMI, and longer study periods should be considered in future studies.

CONCLUSION

This study showed that high and moderate RT in individuals with and without iron stores deficient leads to a significant decrease in hepcidin levels and TNF-α, but just high intensity of RT significantly decreases in IL-6. In conclusion, it seems that doing RT may have positive and anti-inflammatory effects in individuals with and without iron stores deficient.

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Conflicts of interest

There are no conflicts of interest.

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