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Effect of regular Exercise and iron injection on iron status of anemic obese male rats.

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Abstract

Many animal studies have reported an association between obesity and reduced iron storage. Systemic chronic inflammation induced by obesity and higher hepcidin levels has been suggested as a reason for the iron deficiency that occurs with obesity. Hepcidin is a master regulator of systemic iron homeostasis. Hepcidin is a peptide hormone, produced mainly from the liver. It inhibits intestinal iron absorption. The hepatic production of hepcidin is up-regulated by pro-inflammatory cytokines. An exercise is an effective approach for controlling obesity. Several studies showed that exercise reduced body weight. So this study hypothesized that regular exercises may be used as an adjuvant line in treating iron deficiency associated with obesity via reducing serum hepcidin. **Materials and methods:** 75 young male albino rats were categorized into 5 groups. Obesity was induced by high-fat diet (HFD) for 3 months in Group II, III, IV, and V. After 12 weeks, Rats of GIII and GV were injected once every 2 days with iron-III poly-maltose 50 ml/kg for 2 weeks. treadmill regular exercise was performed for 8 weeks for rats of GIV and GV. Measurement of body weight was done after 3 months of HFD and at the end of the experiment. Biochemical analysis was done for serum iron and serum hepcidin. **Results:** Measurement of body weight showed that exercise has a decreasing effect on body weight in obese rats. Exercise decreased serum hepcidin levels in obese rats and increased serum iron. **Conclusion:** Exercise decreases serum hepcidin level also improves the serum iron level in obese rats.

Keywords: Exercise, hepcidin, obesity, serum iron.

Introduction:

Obesity is a common disease defined by an excess of body fat to the extent that health is negatively affected **(19)**. Within only a few years, obesity had become a serious health problem **(19).** Also; Iron deficiency is another common problem in many countries. Many animal and clinical studies have showed an association between overweigh and reduced iron storage **(24).** Systemic low-grade inflammation resulted from obesity and increase hepcidin levels have been suggested as a reason for iron deficiency (ID) that occurs with obesity **(21).**

There are minimally three mechanisms of (ID) that occur with obesity have been proposed and include: (i) decrease iron intake **(29)**; (ii) increased body mass leading to increase iron demand **(2);** and (iii) systemic chronic low-grade inflammation that occurs with obesity **(6).** Hepcidin is the main hormone that regulates systemic iron homeostasis. Hepcidin is a peptide hormone, produced mainly from the hepatocytes. It inhibits iron absorption in the intestine and reduces the iron release from macrophages, the hepatic production of hepcidin is induced by pro-inflammatory cytokines and it is inhibited by (ID) **(12).**

In overweighed individuals, fatty cells are a major source that releases pro-inflammatory cytokines **(17).** Fatty tissue, particularly visceral fatty tissue, is the main source of pro-inflammatory cytokines secretion and a possible reservoir for pro-inflammatory molecules that contribute many diseases. The production of cytokines by fatty tissue is elevated in the presence of total and abdominal adiposity **(15).**

Endothelial abnormalities is associated with obesity and result in vascular damage with the accumulation of phagocytes in the subintimal space and release of many inflammatory cytokines **(22).**

Many studies reported that exercise reduced body weight and adipose mass **(4).** So this work hypothesized that regular exercises may be used as an adjuvant line in treating iron deficiency associated with obesity via reducing serum hepcidin.

Aim of the work

-To evaluate the effect of obesity on an iron level and hepcidin level.

-To evaluate the effect of exercise training and/or iron injection on the iron status of obese male rats.

Materials and methods

Animals

Seventy-five male Wister albino rats, average age about 3-4weeks and weight 130 – 150 g, were housed in groups of four in metal box cages (20 x32 X20 cm) at normal light-dark cycle and room temperature. Rats were obtained from the faculty of Science, Sohag University.

Materials of the study

-Iron-III poly-maltose (Xiamen Vastland Chemical Co., Ltd).

-Hepcidin Elisa Kits (Cat.No: MBS103477 San Diego (USA)): to measure the hepcidin concentration in the serum.

Method of the study

This work was carried out underline the guidelines of the University Animal Ethics and approved by the Research Ethics Committee considering care and use of laboratory animals.

The 75 rats were randomly divided into five equal groups (n=15):

Group I (GI): were used as a control group. Rats were fed a control diet (25 gram approximately of the ordinary feeds for each rat per day) with water all over the experiment **(28)**. After 12 weeks, rats were injected intramuscular (IM) once every 2 days with physiological saline (0.9% NaCl) at a dose of 50 ml/kg for 2 weeks.

Group II (GII): were fed a high-fat diet (HFD) with water all over the study **(28).** After 12 weeks, rats were injected (i.m) once every 2 days with physiological saline (0.9% NaCl) at a dose of 50 ml/kg for 2 weeks.

Group III (GIII): were fed HFD with water **(28).**After 12 weeks, Rats were injected (i.m) once every 2 days with iron-III poly-maltose 50 ml/kg for 2 weeks
(**12**).

Group IV (G IV): were fed HFD with water daily during all the study**(28)** then after 12weeks, treadmill regular exercise was performed for 8 weeks.

Group (GV): were fed HFD with water daily all over the study **(28)** then after 12weeks, Rats were injected (i.m) with iron-III poly-maltose (50 ml/kg) every 2 days for 2 weeks**(11)** with performing treadmill regular exercise for 8 weeks.

Measurement of body weight was done after 3 months of high-fat diet and at the end of the experiment.

Exercise Program

During the period of 1st 4weeks of the programme, exercise was done 5 days/week for 40 minutes /day with a 5 minute warm-up at a speed of 2 m/min, and was followed by 30-minute main exercise at a speed of 8 m/min, and was ended by 5 minutes final cool-down session at a speed of 5 m/min. And then from 5 - 8 weeks, exercise was done five days per week for 60 minutes per day with a 10 minutes warm-up at a speed of 8 m/min, a 40-minute main exercise was at a speed of 14 m /min, with a 10 minute-final cool-down at a speed of 11 m/min. Treadmill exercise was performed where the slope was fixed to zero degrees **(28).**

Collection of samples

After the experiment, the rats were anesthetized with thiopental Na (40 mg/kg) intraperitoneally. Intracardiac samples of blood were being collected, centrifuged to separate the serum, and then the serum was stored at -20 ̊c until the time of biochemical analysis. Biochemical analysis was done for serum iron and serum hepcidin.

Statistical analysis

All of the data are expressed as the means ± standard deviation. Group comparisons were performed using one way ANOVA test and T-test. P-value less than 5% was considered to be significant.

Results:

Effect of iron injection on body weight

IM iron injection to rats of GIII resulted in a statistically non-significant change of body weight when compared with GII (table1).

Effect of Exercise on body weight

Measuring the bodyweight of the rats of GIV at the end of the study showed that the exercise had a decreasing effect on body weight. After 2 months of exercise, there was a statistically significant decrease in the bodyweight of G IV when compared with G II ($p<0.05$) (table 1).

Effect of combined exercise and iron injection on body weight

The bodyweight measurement of GV at the end of the study showed that the combined exercise and iron injection had a statistically significant decrease in body weight when compared with G II($p<0.05$).(table1)

Results of biochemical analysis

Effect of exercise and/ or iron injection on serum level of iron

The results of this study showed that exercise and/or iron injection caused a statistically significant increase in the serum level of iron. GII showed a statistically significant low level of serum iron when compared with GI ($p<0.05$). IM injection of iron to GIII resulted in a statistically significant elevated level of serum iron at the end of the experiment when compared with GII($p<0.05$). Interestingly, also exercise produced a statistically significant high level of serum iron in rats of GIV when compared with rats of GII ($p<0.05$). Combined exercise and iron injection resulted in a statistically significant high level of iron in rats of GV when compared with rats of GII (p<0.05).

Results of one way ANOVA and post hoc test done for GII, GIII, GIV, and GV showed that there was a statistically significant high level of serum iron in GIII and GIV with statistically significant more high level of serum iron in GV(table 2). There was a significant negative correlation between serum iron and body weight (r=-0.477). As correlation is significant at 0.01.

Effect of exercise and/ or iron injection on serum level of hepcidin.

Results of this study showed that exercise and combined exercise and iron injection caused a statistically significant decrease in the serum level of hepcidin. GII showed a statistically significant high level of serum hepcidin when compared with GI (p<0.05). IM injection of iron to GIII didn't have a decreasing effect on the level of serum hepcidin at the end of the experiment. Interestingly, exercise produced a statistically significant low level of serum hepcidin in rats of GIV when compared with rats of GII (p<0.05). Combined exercise and iron injection resulted in a statistically significant low level of hepcidin in rats of GV when compared with rats of GII (p<0.05). Results of one way ANOVA and post hoc test done for GII, GIII, GIV, and GV showed that there was a statistically significant low level of serum hepcidin in GIV and GV when compared with rats of GII (table 2). There was a significant positive correlation between serum hepcidin and body weight (r=0.981). Also, There was a significant negative correlation between serum iron and serum hepcidin (r=-0.465). As correlation is significant at 0.01.

Table (1): Average of body weight at the start, after 3months and at the end of the experiment in all groups of the study.

Groups	GI	GII	GIII	GIV	GV
Initial weight	147.85±9371	147.55±8.929	147.75± 9.324	148.10± 8.416	148.25± 9.210
After 3 months	187.65±9.178	314.3±6.82 #	#315.10±6.96	#314.85±7.27	314.50±7.186 #
At the end of experiment	223.95±5.633	542.75±18.761 #	528.55±13.953 #	247.45±10.475 *	261.50±8.859 *

-Data are expressed as mean ± standard deviation.
-P-Value was calculated sig (p<0.05).
#Significant when compared with GI done by t test
**significant when compared with GII done by ANOVA.*

Table (2): Average of serum level of iron and hepcidin at the end of experiment in all groups of the study

Groups / parameters	GI	GII	GIII	GIV	GV
Serum level of iron (µg/dl)	240.45±21.833	147.95±21.368 #	289.35±14.195 *	289.45±22.385 *	308.65±15.253 *
Serum level of hepcidin (mg/dl)	70.40±5.471	142.25±6.695 #	140.60±7.287 #	75.05±6.863 *	76.95±6.151 *

-Data are expressed as mean ± standard deviation.
-P- Value was calculated sig (p<0.05).
#Significant when compared with GI done by t test
**significant when compared with GII done by ANOVA.*

Discussion

Obesity is a major risk factor for several depleting diseases such as diabetes mellitus, cardiac problems, and cancer that are now dramatically increased in many countries. Obesity is associated with an excessive increase in visceral fatty tissue mass. White fatty tissue secretes many pro-inflammatory and anti-inflammatory factors (**18).**

An increase in body fat tissue may be related not only to energy intake and energy output in humans but also to the quality of diet, especially (HFD), which may lead to many metabolic disturbances such as overfeeding in humans, decreased lipolytic action in fat tissue, decrease in leptin secretion and/or decrease sensitivity, hypothalamic neuron degeneration, insulin resistance, and overweight **(7).**

In this present study, a model of obesity was designed by using high-fat diets (HFD)for3months

In this study, there was a statistically significant decrease in the serum iron level in GII when compared to group I. This agreed with **(2)** who reported that increases BMI affected the iron status of obese patient by showing low iron levels and high ferritin levels in over-weighted patients than the normal weight subjects. In contrast, **(16)** in their study, there wasn't a clear difference in iron status with different levels of BMI among males and females. Although, many studies have reported that there was a link between obesity and (ID), the mechanisms of this are still confused.

Interestingly, the level of serum hepcidin was significantly increased in GII when compared to GI. These data are following **(23)** who showed that there was a statistically significantly higher serum hepcidin level in Overweight individuals when compared to those with normal (BMI) and suggest that production of hepcidin by fatty cells may be higher in obesity. In contrast, **(26)** showed that the adipose tissue may produce very little serum hepcidin levels compared to the liver production of hepcidin

This investigation found a strong positive relationship between BW and hepcidin levels, also found a strong negative relationship between hepcidin levels and iron levels. This agreed with **(1)** who confirmed that obese children had high hepcidin levels and low serum iron when compared to lean children. In contrast, **(23)** supposed that serum hepcidin does not contribute to the development of (ID) anemia in obese children.

Excess accumulation of fatty tissue is characterized by a status of low-grade chronic inflammation. This leads to the production of inflammatory cytokines, such as interleukin 6, which is well known to stimulate hepatic hepcidin synthesis **(9)**. Hepcidin decrease the release of iron from macrophages and reduced intestinal iron absorption resulted in less iron is released and more iron stores, explaining the status of low serum iron in the over-weighted individuals **(20).**

This study investigated the effect of exercise and\or iron injection on body weight and the results showed that exercise has a decreasing effect on body weight.

After 2 months of regular exercise, it was a statistically significant decreasing effect on BW in a group (IV) and group (V) when compared with the group (II). This agreed with **(14)** who showed that regular exercise is essential for weight control and weight loss.

After 2 weeks of iron injection, iron did not alter the (BW) in the GIII. These results are following **(11)** who showed that systemic iron supplementation did not have a decreasing effect on body weight and adiposity.

This study investigated the effect of exercise and\or iron injection on serum level of iron. The results of this study showed that exercise and/or iron injection caused a statistically significant increase in the serum level of iron.

After 2 months of regular exercise, interestingly, exercise produced a statistically significant high level of serum iron in rats of a group (IV) when compared with rats of a group (II). These results are following **(8)** who confirmed that physical exercise had an increasing effect on iron profiles and decreasing effect on levels of inflammatory markers when compared with the control group and the mechanism by which regular exercise increases iron levels occur by the decreasing the adipose mass with a significant decrease in inflammation and reported that the decrease in IL-6 led to decrease in hepcidin level **(8).** This study did not evaluate pro-inflammatory markers. In contrast**, (5)** reported that (ID) anemia is likely to be more in athletes than in sedentary individuals.

After 2 weeks of iron injection, the IM injection of iron to GIII resulted in a statistically significant elevated level of serum iron at the end of the experiment when compared with GII. These results are following **(11)** who confirmed that iron supplementation increased serum levels of iron and decreased iron-binding capacity (IBC). Combined exercise and iron injection resulted in a statistically significant high level of iron in rats of GV when compared with rats of GII.

In this work, we investigated the effect of exercise and/ or iron injection on the serum level of hepcidin. The results of this study showed that exercise caused a statistically significant decrease in the serum level of hepcidin.

After 2 months of regular exercise, interestingly, exercise produced a statistically significant low level of serum hepcidin in rats of GIV when compared with rats of GII. This agreed with **(3)** who showed that physical exercise reduces hepcidin levels and increases iron levels. In contrast**,(13)** reported that high intense exercise was associated with marked high levels of hepcidin and marked increase in Interleukin-6. Moreover**,(13)** reported that acute high intense exercise increases Interleukin-6 and hepcidin levels 3hours after the exercise. The mechanism by which physical regular exercise reduced serum level of hepcidin is still unclear. **(8)** showed that long term regular exercise reduced BMI and improved inflammatory status, particularly in IL-6, which reduced hepcidin levels and impairment in iron status.

After 2 weeks of iron injection, the IM injection of iron to GIII didn't have a decreasing effect on the level of serum hepcidin at the end of the experiment. These results are the following **(11)** who found that iron injection did not affect systemic hepcidin synthesis.

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