

## Association of Hematological Parameters with Obesity- Induced Inflammation Among Young Females in Ahvaz, South-West of Iran

### Abstract

**Background:** Iron deficiency is prevalent in overweight and obese individuals and may be induced by adiposity-related inflammation that affect iron metabolism. **Objective:** The objective of this study was to investigate the relationship between hematological parameters and obesity-induced inflammation among young females. **Methods:** A total of 170 young women (aged between 18-35 years) participated in this cross-sectional study. Obesity was assessed by BMI (body mass index), WHR (waist to hip ratio), and body fat percentage. Inflammatory and hematological parameters including hs-CRP (high-sensitivity C-reactive protein), serum Fe, hemoglobin, ferritin, transferrin, TIBC (total iron binding capacity) were measured. Dietary intakes of some nutrients (total iron, proteins, calcium, and vitamin C) were assessed according to BMI, WHR and fat mass categories. **Results:** Serum iron were negatively correlated with BMI ( $P = 0.045$ ,  $r = -0.154$ ) and hs-CRP ( $P = 0.032$ ,  $r = -0.165$ ). Hemoglobin were also negatively correlated with BMI ( $P = 0.043$ ,  $r = -0.155$ ). A significant correlation was also shown between WHR with transferrin ( $P = 0.034$ ,  $r = 0.163$ ) and TIBC levels ( $P = 0.035$ ,  $r = 0.162$ ), hs-CRP was positively correlated with BMI ( $P = 0.014$ ,  $r = 0.183$ ), WHR ( $P = 0.009$ ,  $r = 0.202$ ) and body fat percentage ( $P = 0.037$ ,  $r = 0.353$ ). Dietary intakes did not differ significantly among BMI, WHR and fat mass categories ( $P > 0.05$ ). **Conclusions:** Obesity-induced inflammation, regardless of dietary intake of iron, can lead to iron deficiency. Therefore, weight control, especially in obese subjects is necessary to prevent iron deficiency and anemia.

**Keywords:** Hematological parameters, inflammation, iron deficiency, obesity, young female

### Introduction

Iron deficiency is the most common and widespread nutritional disorder in the world. Over 30% of the world's populations are anemic, many due to iron deficiency.<sup>[1]</sup> In Iran, 23.7% of adolescent girls (14-20 years) and 40.9% of young women (18-25 years) have iron deficiency, and 12.2% and 8.3% have iron deficiency anemia, respectively.<sup>[2,3]</sup> Iron deficiency is characterized by hemoglobin concentrations greater than or equal to 12 g/dl and serum ferritin levels less than 12 ng/ml, however, iron deficiency anemia (IDA) is defined as hemoglobin concentrations less than 12 g/dl and serum ferritin levels less than 12 ng/ml.<sup>[3,4]</sup> Iron deficiency and its following anemia can lead to physical weakness, fatigue, shortness of breath, dizziness, headache, pale skin, weakened immune system, higher prenatal mortality and low birth weight and premature infants.<sup>[3,5,6]</sup> Poor

diet and low intake of iron are the main causes of iron deficiency among adolescent girls and young women.<sup>[1]</sup> Moreover, several studies proposed that obesity-associated inflammation might be linked to iron deficiency.<sup>[4]</sup> Some evidence suggested that overweight and obese individuals have a greater chance to be developed iron deficiency anemia.<sup>[5,7]</sup> However, the etiology of iron deficiency following obesity remains unclear. In this regards, poor dietary iron intake, deficient iron stores and increased iron requirements in obese individuals are suggested for higher susceptibility of obese population to iron deficiency.<sup>[8]</sup> However, other studies indicated that iron deficiency in obese individuals were not due to low dietary intake or high dietary requirement of iron;<sup>[9,10]</sup> and most likely, adiposity-related inflammation in obese individuals was responsible for iron deficiency.<sup>[7]</sup> It was suggested that obesity-induced inflammation increased hepcidin concentrations, consequently, reduced intestinal iron

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absorption or inhibited iron uptake by ferroportin, a cellular iron exporter, from liver cells, macrophages and enterocytes.<sup>[11-14]</sup>

On the other hand, the prevalence of overweight and obesity are rapidly expanding worldwide in recent years. In Iran, obesity prevalence was estimated 19.4% in 2011; so that 26.5% of women and 12.4% of men were obese. The prevalence of overweight in Iranian population was even far more. In addition, in developing countries, such as Iran, that undergoing the nutrition transition, there are double burden of malnutrition (coexistence of over- and under nutrition).<sup>[15]</sup> Iron deficiency and anemia are the most prevalent nutritional deficiencies among young women.<sup>[2,3]</sup> Therefore, regarding the coexistence and growing prevalence of these two nutritional disorder (obesity and iron deficiency) in Iran, this question is asked that whether there is a link between obesity and iron deficiency or not. Moreover, due to the important role of young women's health in the health of society, this study was designed to evaluate the association between obesity, inflammation and iron status in young women in Ahvaz, southwest of Iran.

## Methods

### Subjects

In this cross-sectional study, 170 young women were recruited randomly from university students, at Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz city, in the southwest of Iran from June to September 2015. All participants were single healthy women, aged 18-35 years, with no chronic disease (diabetes, cardiovascular disease, inflammatory diseases, parasitic diseases and hemorrhoid) and had regular menstrual cycle, with no amenorrhea or dysmenorrhea, were not participating in weight loss regimes or professional sports program during the previous 6 months, and were not taking vitamin, mineral, laxative, hormone medications, or any supplements that affect iron metabolism, at least 6 month before sampling.

The number of participants calculated by using the NCSS software was 170 according to the existence of 80 participants with iron deficiency, and also based on the prevalence of iron deficiency anemia in the previous study<sup>[3]</sup> that was 47%.

The current study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Study project number: NRC-9301, ethical approval study: ajums.REC.1393.29). This approval was presented to the participants, and before the participation in the study, an informed written consent was obtained from each participant.

### Anthropometric, dietary intake and physical activity assessments

Participants completed a questionnaire to determine their demographic characteristics. Participants' weight and height

were also measured using a balanced scale (Seca, model 760, Hamburg, Germany) in light clothing and without shoes. BMI was calculated as weight in kilograms divided by the square of the height in meters ( $\text{kg}/\text{m}^2$ ). Waist and hip circumferences were measured to the nearest 0.1 cm using a flexible metric tape in the subject with a standing position. Waist circumference was determined around the abdomen at the level of the umbilicus. Hip circumference was determined at the level of the maximum extension of the buttocks posteriorly in a horizontal plane. Then, WHR was calculated as waist circumference (cm) divided by hip circumference (cm). Body fat was analyzed with bioelectric impedance (BI) method. An Omron HBF 306 body fat monitor (Omron Co., Ltd, Japan) was used. In this study, three indices, including BMI, WHR and body fat mass percentage, were used to determine obesity. Women were classified by BMI categories as underweight: BMI less than  $18.5 \text{ kg}/\text{m}^2$ , normal weight: BMI 18.5 to 24.9, overweight: BMI 25 to 29.9, obese: BMI over 30. The waist to hip ratio is a simple measure of central obesity. In this study, central obesity was defined as a WHR  $\geq 0.8$  (in women). Regarding the fat mass percentage, women with body fat percentage  $\geq 30\%$  were considered as obese.<sup>[16]</sup> Usual dietary intakes were investigated by a validated 168-item semi-quantitative food frequency questionnaire (FFQ).<sup>[17]</sup> Then, N4 (Nutritionist 4) nutritional software was used for determining nutrient compositions of foods. To assess physical activity, short form of the international physical activity questionnaire (IPAQ) was used. Data from the IPAQ was converted to MET-minutes/week using the existing guidelines.<sup>[18]</sup>

### Laboratory analysis

After a 12 h overnight fasting, blood samples were obtained for biochemical and haematological screening tests. A professional staff performed venepuncture, using two types of test tubes, one of the tubes contained EDTA, to obtain 10 ml blood. The EDTA-blood was transferred to the Laboratory of Nutrition and Metabolic Diseases Research Center at Ahvaz University, on the same day of collection, for determining the haematological parameters, including erythrocyte count, Hb and mean corpuscular volume. The remaining was collected in plain test tubes for the preparation of serum. Serum Fe and total iron binding capacity (TIBC) levels were measured by colorimetric assays. Transferrin was measured by immunoturbidimetric assay. Serum ferritin concentration was measured by enzyme-linked immunosorbent assay (ELISA) using commercial kit (Lake Forest, CA 92630 USA). Serum hs -CRP level was also measured by ELISA method (Labor Diagnostika Nord, Germany).

### Statistical analysis

Statistical analyses were performed by using SPSS version 16.0 (SPSS Inc, Chicago, Illinois) software. Kolmogorov-Smirnov's test was used to check the normality

of data. All variables had normal distribution. Descriptive analyses were computed for each of the variables. The correlations between variables were controlled for the effects of confounders (age, energy intake, and physical activity levels) by Partial correlation. One-way ANOVA test was also used for comparing the variables in different categories of BMI, WHR, and body fat percentage. *P* value less than 0.05 was considered significant.

## Results

In this study, 170 young females with an average age of  $23.9 \pm 3.7$  years were studied. The mean  $\pm$  standard deviation (SD) of BMI and body fat mass percentage was  $23.5 \pm 3.3$  and  $33.9 \pm 4.8$ , respectively [Table 1].

In the present study, BMI, WHR and body fat mass percentage were used to determine obesity. Accordingly, the absolute and relative frequencies of participants in different categories are shown in Table 2.

The results showed that a total of 35.29% (N = 60) of participants were overweight and obese based on BMI criteria. According to WHR criteria, 32.4% (N = 55) of females were characterized as abdominal obesity. However,

**Table 1: Demographic and anthropometric characteristics of the study participants**

Variables	Mean $\pm$ SD	Minimum	Maximum
Age (year)	23.9 $\pm$ 3.7	18.0	35.0
Weight (kg)	61.1 $\pm$ 9.0	42.0	86.5
BMI (kg/m <sup>2</sup> )	23.5 $\pm$ 3.3	17.0	39.5
WHR	0.85 $\pm$ 0.05	0.62	0.94
BF (%)	33.9 $\pm$ 4.8	12.0	45.5
IPAQ (met-min/wk)	2092 $\pm$ 58.42	2035	2152

\*BMI=Body mass index, WHR=Waist to hip ratio, BF=Body fat; IPAQ=International Physical Activity Questionnaire

**Table 2: Absolute and relative frequency of participants in various categories of BMI, WHR, BF**

Variables	Absolute frequency (N)	Relative frequency (%)
BMI categories		
Underweight (BMI $\leq$ 18.5)	6	3.53
Normal (BMI: 18.5-24.9)	104	61.17
Overweight (BMI: 25-29.9)	55	32.35
Obese (BMI $\geq$ 30)	5	2.94
total	170	100
WHR categories		
Normal (WHR <0.8)	115	67.6
Central obesity (WHR $\geq$ 0.8)	55	32.4
total	170	100
BF categories		
Normal (<30%)	21	12.4
Obese ( $\geq$ 30%)	149	87.6
Total	170	100

\*BMI=Body mass index, WHR=Waist to hip ratio, BF=Body fat

87.6% (N = 149) of participants were obese in this study according to body fat mass percentage. In this study, there was not statistically significant difference in dietary intakes of iron and other relevant nutrients among women in different categories of obesity (*P* > 0.05). In Table 3, dietary intakes of iron, vitamin C, calcium and protein were shown in different categories of obesity.

In Table 4, hematological indices of participants were compared in terms of different categories of obesity. In the present study, the mean concentration of serum Fe, hemoglobin and hematocrit were significantly different in various categories of BMI (*P* = 0.026, *P* = 0.010 and *P* = 0.031, respectively). As well as, significant differences were observed in serum transferrin and TIBC levels among different categories of WHR (*P* = 0.040, *P* = 0.034 respectively). As, women with central obesity have significantly higher levels of transferrin and TIBC compared to normal women (*P* = 0.040 and *P* = 0.034, respectively). The results also showed significant differences in serum levels of iron and ferritin among women with various categories of body fat percentage (*P* = 0.041 and *P* = 0.037, respectively).

The relationship between levels of hematological parameters with criteria used for defining obesity (BMI, WHR and BF) and serum hs-CRP concentrations are shown in Table 5. Based on the results, a significant negative correlation was observed between BMI with serum iron (*P* = 0.045, *r* = -0.154) and hemoglobin levels (*P* = 0.043, *r* = -0.155). A significant correlation was also shown between WHR with transferrin (*P* = 0.034, *r* = 0.163) and TIBC levels (*P* = 0.035, *r* = 0.162). A significant negative correlation was also shown between body fat mass percentage with serum levels of iron (*P* = 0.020, *r* = -0.749) and ferritin (*P* = 0.029, *r* = -0.711). Serum levels of hs-CRP and iron were also negatively correlated in this study (*P* = 0.032, *r* = -0.165).

The relationship between obesity (defined by BMI, WHR and percentage body fat) and serum hs-CRP levels are shown in Table 6. In the present study, a statistically significant and positive relationship was observed between hs-CRP with BMI (*P* = 0.014, *r* = 0.183), WHR (*P* = 0.009, *r* = 0.202) and body fat percentage (*P* = 0.037, *r* = 0.353).

## Discussion

In this study, 170 young women with an average age of  $23.9 \pm 3.7$  years were studied about iron status and its relation to obesity and obesity-induced inflammation. According to the results, the mean serum iron, hemoglobin and hematocrit levels were significantly lower in overweight and obese subjects (BMI >25) compared to non-obese subjects. The results also showed that women with higher body fat mass percentage (BF  $\geq$ 30) have significantly lower levels of serum iron and ferritin. Similarly, in a study that was conducted by Yanouf *et al.*, in

**Table 3: The mean and standard deviation of some dietary intakes in different categories of BMI, WHR, BF.**

Variables	Energy (kcal/day)	Total iron (mg/day)	Vitamin C (mg/day)	Calcium (mg/day)	protein (gr/day)
BMI categories					
Underweight (BMI ≤18.5)	2139±369	13.89±3.08	92.39±20.33	204.4±120.0	72.36±18.98
Normal (BMI: 18.5-24.9)	2360±847	16.44±5.92	114.94±59.50	330.6±145.3	85.13±21.55
Overweight (BMI: 25-29.9)	2071±592	14.33±4.28	104.00±24.51	301.8±131.4	76.33±26.21
Obese (BMI ≥30)	2136±275	18.21±2.51	94.53±21.49	120.2±68.1	84.70±20.19
P value	0.138	0.202	0.678	0.378	0.279
WHR categories					
Normal (WHR <0.8)	2323±833	16.05±5.54	110.47±36.65	309.1±128.2	82.42±32.00
Central obesity (WHR ≥0.8)	2103±543	15.02±4.72	109.03±25.04	297.5±125.6	80.60±23.39
P value	0.048	0.102	0.510	0.701	0.119
BF percentage					
Normal (<30%)	2284±673	15.52±4.67	103.89±24.34	303.4±134.5	84.22±22.85
Obese (≥30%)	2247±770	15.74±5.02	110.86±32.30	311.4±126.7	81.49±29.02
P value	0.330	0.390	0.850	0.540	0.774

\* BMI=Body mass index, WHR=Waist to hip ratio, BF=Body fat

**Table 4: The mean and standard deviation of hematological parameters in different categories of BMI, WHR, BF**

Variables	Serum Fe (µg/dl)	Hemoglobin (g/dl)	Hematocrit (%)	Transferrin (mg/dl)	TIBC (µg/dl)	Ferritin (ng/dl)
BMI categories						
Underweight (BMI ≤18.5)	111.33±35.33	12.40±0.66	38.26±1.45	247.40±30.46	309.0±38.22	22.43±6.31
Normal (BMI: 18.5-24.9)	94.26±36.07	12.07±0.90	37.77±2.26	252.38±42.41	315.61±53.42	28.44±5.67
Overweight (BMI: 25-29.9)	82.70±35.93	11.58±1.12	36.88±2.82	259.89±42.86	324.74±53.56	28.24±1.71
Obese (BMI ≥30)	85.20±20.58	11.78±0.78	36.22±2.56	274.16±47.20	342.16±58.94	25.28±5.29
P value	0.026*	0.010*	0.031*	0.51	0.53	0.93
WHR categories						
Normal (WHR <0.8)	90.77±35.60	11.96±1.03	37.51±2.47	244.07±34.48	305.25±43.80	26.06±3.11
Central obesity (WHR ≥0.8)	91.05±37.18	11.83±0.92	37.52±2.48	264.06±44.56	329.92±55.63	32.28±5.52
P value	0.96	0.41	0.98	0.040*	0.034*	0.11
BF categories						
Normal (<30%)	91.38±35.99	12.12±1.40	37.76±2.97	256.13±41.23	320.15±51.67	29.73±8.96
Obese (≥30%)	87.14±36.85	11.89±0.92	37.48±2.39	268.0±50.60	334.61±63.27	27.83±3.34
P value	0.041*	0.31	0.62	0.23	0.24	0.037*

BMI=Body mass index, WHR=Waist to hip ratio, BF=Body fat, TIBC=Total Iron Binding Capacity. \*Results are statistically significant

**Table 5: Association between hematological parameters, obesity (defined by BMI, WHR and percentage body fat) and hs-CRP<sup>1</sup>**

Variables		BMI (kg/m <sup>2</sup> )	WHR	BF (%)	hs-CRP (mg/L)
Serum Fe (µg/dl)	r	-0.154	-0.240	-0.794	-0.165
	P	0.045*	0.754	0.020*	0.032*
Hemoglobin (g/dl)	r	-0.155	-0.077	-0.137	-0.323
	P	0.043*	0.318	0.076	0.076
Hematocrit (%)	r	-0.057	-0.011	-0.222	-0.401
	P	0.458	0.891	0.094	0.065
Transferrin (mg/dl)	r	0.180	0.163	0.272	0.124
	P	0.159	0.034*	0.085	0.118
TIBC (µg/dl)	r	0.105	0.162	0.283	0.131
	P	0.174	0.035*	0.083	0.106
Ferritin (ng/dl)	r	-0.025	-0.148	-0.711	-0.169
	P	0.747	0.055	0.029*	0.106

BMI=Body mass index, WHR=Waist to hip ratio, BF=Body fat; hs-CRP=High sensitive C reactive protein; TIBC=Total Iron Binding Capacity. \*Results are statistically significant, <sup>1</sup>Controlling for the effects of age, energy intake and physical activity levels by partial correlation



**Table 6: Association between obesity (defined by BMI, WHR and percentage body fat) and serum hs-CRP levels<sup>1</sup>**

Variables		hs-CRP (mg/L)
BMI (kg/m <sup>2</sup> )	r	0.183
	P	0.014*
WHR	r	0.202
	P	0.009*
BF (%)	r	0.353
	P	0.037*

BMI=Body mass index, WHR=Waist to hip ratio, BF=Body fat, hs-CRP=High sensitive C reactive protein. \*Results are statistically significant, <sup>1</sup>Controlling for the effects of age, energy intake and physical activity levels by partial correlation

2007, obese subjects had lower iron levels than non-obese subjects.<sup>[11]</sup> In 2011, Cepeda Lopez and colleagues also reported that the prevalence of iron deficiency in obese women and children is 2-4 times more than the subjects with normal weight.<sup>[19]</sup>

On the other hand, in terms of iron deficiency, the levels of transferrin and TIBC raised,<sup>[4]</sup> therefore, as expected, in this study, women with abdominal obesity (WHR  $\geq 0.8$ ) had also significantly higher levels of transferrin and TIBC. In this regard, the investigators stated that iron status is predicted by C-reactive protein concentrations independently of differences in dietary iron intake.<sup>[19]</sup> According to several studies, obesity and having higher fat reserves than the normal values is considered as a chronic systemic inflammatory condition that is consequently associated with increased secretion of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6 and CRP.<sup>[14,19]</sup> In the present study, after controlling for confounders (age, energy intake, and physical activity), serum hs-CRP levels were positively associated with all three criteria used to define obesity (BMI, WHR, and BF). Similar results have been reported in other studies.<sup>[11,14,19,20]</sup> These results could indicate obesity induced chronic inflammation in overweight and obese subjects. The positive association of CRP with obesity and its negative correlation with serum levels of iron have been reported in some studies.<sup>[7,11]</sup> In this study, hs-CRP levels were negatively associated with iron concentrations. This could indicate that in obese subjects, increased concentrations of pro-inflammatory cytokines such as CRP, may induce hepcidin production by the liver and adipose tissue.<sup>[19,20]</sup> Hepcidin is a peptide hormone that plays a key role in the regulation of iron homeostasis. In physiological conditions, hepcidin is mainly secreted by the liver, however, in inflammatory conditions, such as obesity, adipose tissue also has an important role in its production. In some studies, it is suggested that hepcidin concentration increases in response to inflammation.<sup>[21-23]</sup> Under the conditions in which the hepcidin level is high, such as inflammation, serum iron falls due to iron trapping within macrophages and liver cells it reduces intestinal

iron absorption. These conditions typically lead to anemia due to an inadequate amount of serum iron required for developing the red cells.<sup>[24-26]</sup>

Interestingly, our results also showed that obese women with higher fat mass (BF  $\geq 30$ ) have higher levels of hs-CRP. Consequently, these subjects also had lower iron and ferritin concentration [Table 4]. These results confirmed the role of obesity-induced inflammation in the etiology of iron deficiency. However, because of some limitations, hepcidin concentration was not assessed in this study. Therefore, it is unclear whether obesity-induced inflammation increased iron deficiency by increasing hepcidin concentrations or not. Hence, future studies are required to determine the exact mechanisms of the association between obesity-induced inflammation and hematologic parameters.

However, the most important strength of this study was to investigate the association of hematologic indices with three different criteria to define obesity, and some interesting results were obtained in this regard. In order that some indices of iron deficiency solely correlated with body fat mass percentage, and not necessarily related to BMI. This could be confirmed the limitation of BMI to define obesity as discussed in several studies.<sup>[16]</sup>

Dietary intakes of iron and other nutrients influencing on iron status (protein, calcium and vitamin C) were not statistically different between normal and obese participants in this study. However, obese subjects had lower levels of some hematologic indices compared to non-obese subjects. Similar results have been reported by others.<sup>[7,19]</sup> Therefore, it seems that in obese patients, iron deficiency is not necessarily due to poor dietary intake or inappropriate dietary patterns; and obesity-related inflammation and consequently elevated levels of hepcidin may be responsible for disturbing iron status in obesity. However, some studies have shown conflicting results with the present study. In Qin *et al.*, study in 2013, overweight and obese women had higher dietary iron intake. It was also demonstrated that overweight and obese women were less likely to develop iron deficiency anemia compared to normal weight subjects.<sup>[25]</sup> In another study by AlQuaiz *et al.*, inadequate intake of meat were associated with increased risk of anemia, whereas having higher BMI was associated with reduced risk of anemia in childbearing age women in Riyadh, Saudi Arabia.<sup>[27]</sup> However, in two recent studies, ferritin values were used for defining iron deficiency anemia. Since, various factors are involved in the etiology of iron deficiency, it seems that the diagnosis of iron deficiency anemia should not be limited to ferritin concentration because some evidence indicates that ferritin reserves may even increase in chronic inflammation. Therefore, future studies to determine the exact mechanisms of the effect of obesity-induced inflammation on iron status are suggested.

## Conclusions

Obesity and consequently chronic inflammation, regardless of dietary intake of iron, correlated with some hematologic parameters. Therefore, it seems that having a normal weight in addition to consumption of adequate iron can be effective to prevent iron deficiency. However, more studies are suggested to clarify the various aspects of the relationship between obesity and iron deficiency in both sexes.

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## Conflict of interest

There are no conflicts of interest.

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