Progress in Nutrition 2019; Vol. 21, Supplement 1: 50-56 DOI: 10.23751/pn.v21i1-S.5503

Original Article

Effect of a weight loss intervention on iron parameters in overweight and obese Turkish women

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Summary. *Background/aims:* To examine changes in body weight and iron parameters of overweight and obese premenopausal women who participated in a weight loss intervention. *Methods:* A total of 147 overweight or obese women, aged 20-49 years, were included in this study. Subjects were divided into two groups at the baseline of study. All women underwent a 3 month intervention, a nutritional weight loss program. The first group included women with low hemoglobin levels, as the second group consisted of women with normal hemoglobin levels. Biochemical, and anthropometric parameters were measured, and dietary intake was recorded at the baseline and end of the study. *Results:* After the intervention, first group had 10.1% weight loss, whereas the second group had 10.7%. A statistically significant relationship between body weight loss and C-reactive protein (CRP) levels was determined. Significant decrease was observed on the anthropometric variables, dietary energy, total fat, saturated fatty acids, and carbohydrate intake. Dietary intakes of vitamin C, fiber, iron, and calcium levels increased. *Conclusion:* Weight loss improved the blood iron parameters and anthropometric measurements possibly due to its possitive effect on inflammation.

Key words: iron deficiency, obesity, weight loss intervention

Introduction

Iron deficiency (ID) and iron deficiency anemia (IDA) are global public health problems affecting both developing and developed countries with major consequences on human health (1). Recent studies have reported that obese women are more susceptible to ID than normal-weight women (2,3). Although, the underlying mechanisms is not clear, ID in obese individuals might be caused by lower dietary iron intake, reduced iron absorption in the small intestine, and increased iron requirements as a result of larger blood volume, and body surface area in obese subjects (2,4). In addition, obesity is associated with a chronic low-grade inflammation state which either decreases intestinal iron absorption or increases reticuloendothelial iron sequestration (5).

In a study of Amato et al., weight loss was resulted with significant improvements on the iron status of 15 obese children and adolescents who underwent a 6-month weight loss program (6).

This intervention study was performed on a group of overweight and obese women investigating the effect of a weight loss program on hematological parameters and CRP levels aimed to investigate the link between weight loss and iron status. To our knowledge, this study is performed first time in Turkey. It was hypothesised that a systematic body weight loss would improve the iron status of women.

Methods

Patients

This intervention study was conducted to test the changes on blood iron parameters and inflammation markers among overweight and obese premenopausal women who participated in a weight loss program. This study was conducted in Turkey from March to November 2012. A total of 147 overweight (BMI=25.0-29.9 kg/m^2) or obese (BMI=≥30 kg/m^2) women, aged 20-49 years, referred to the internal medicine, endocrinology, and diet outpatient clinics of the İzmir Bozyaka Training and Research Hospital were recruited in the intervention study.

A 3-month weight loss program was introduced to participants (n=147). The participants were assigned into two following groups: first group (n=101) consisted of women with normal hemoglobin levels (hemoglobin>12.0 g/dL), and the second group (n=46) consisted of women with IDA who haven't received iron treatment. IDA was defined as hemoglobin levels to be under 12.0 g/dL (7).

Criteria for study exclusion were: pregnancy, postmenopause, lactation, low BMI (BMI<18.5 kg/m^2), receiving iron supplementation, history of hemorrhage in the last 6 months, alcohol consumption over 50 g/day, presence of comorbidity, drug abuse, receiving medication affecting body weight, and history of bariatric surgery. Subjects were withdrawn from the study in the presence of a condition that would affect body iron stores.

All subjects gave their written and verbal informed consent and the study was approved by the Ethics Committee of Faculty of Medicine, Hacettepe University, Ankara, Turkey (Approval number, 431-1305). This study was funded by Hacettepe University Scientific Research Unit (Approval number, 012DO6401002).

Dietary intake and determination of nutritional status

All patients consumed a nutritionally balanced (55-60% carbohydrate, 25-30% fat and 12-15% protein) self-selected diet, consisted of common foods and underwent lifestyle modifications. The nutrition program was prepared by a registered dietitian for each participant. Participants were asked to record all foods and beverages consumption within 3 consecutive days. A nutrient database (BeBiS, Ebispro for Windows, Germany; Turkish Version/BeBiS 7) was used to estimate the energy and nutrient intake and results were compared with the Dietary Guidelines of Turkey (8). The amount and portion sizes of foods consumed were measured with 2-dimensional food models and a food atlas including 120 photos of foods covering 3 to 5 different portion sizes (9). The daily dietary intake of total macronutrient and micronutrient were analyzed through the dietary intake data collected for 3 days.

Assessment of biochemical parameters

Red blood cell count (RBC), hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), serum iron, total iron binding capacity (TIBC), ferritin, and transferrin saturation were used to determine biochemical index of iron status, as CRP was used to determine inflammation.

Blood samples were obtained for biochemical and hematological screening tests after an 8 hours of overnight fast. Serum iron levels were measured in morning after an overnight fast using the FerroZine method without depolarization (Roche Diagnostics GmbH, Mannheim, Germany). Transferrin saturation (TSAT) was calculated by the ratio of serum iron to total iron binding capacity (TIBC). Serum ferritin was measured by immunometric assay (IMMULITE 2000, EURO/DPC Ltd, Los Angeles, CA, USA). Hemoglobin and mean corpuscular volume (MCV) were measured using flow cytometry (CELL-DYN® 4000, Abbott Laboratories, Abbott Park, IL, USA). C-reactive protein was measured by a high-sensitivity assay (IMMAGE Immunochemistry Systems, Beckman Coulter Inc., Fullerton, CA, USA).

Anthropometric measurements

Anthropometric measurements were determined according to World Health Organization (WHO) criteria (10). Weight was measured using TANITA TBF 300, Tanita Corp., Tokyo, Japan. Women were asked to avoid intensive exercise and excessive food or liquid intake for 4 hours prior to measurement. Height was measured using a tape measure on women standing barefoot, keeping shoulders in a relaxed position, arms hanging freely and head in Frankfurt horizontal plane. Women were classified according to BMI categories as overweight (BMI=25.0-29.9 kg/m^2) and obese (BMI=≥30 kg/m^2) (10).

Anthropometric measurements, biochemical parameters, and nutrient intake were evaluated at the baseline and after the weight loss program.

Statistical analysis

Statistical analyses were performed by the use of IBM SPSS Statistics Version 15.0 software (SPSS Inc., Chicago, IL, USA). The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. The Wilcoxon test and Paired Samples t test were used to assess differences in anthropometric measurements, dietary intake assessments and biochemical markers between baseline and 3 months. $p< 0.05$ was considered to be statistically significant.

Results

After the weight loss program the BMI of both groups were reduced. A reduction of fat mass was observed in most of the participants who had a weight loss had a weight loss. Three months following the intervention, women with anemia had 10.1% weight loss, while those without anemia had 10.7% (Table 1).

A significant increase in iron, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), ferritin, transferrin saturation (TSAT), and a significant decrease in C-reactive protein (CRP) and total iron binding capacity (TIBC) was observed in the first group ($p<0.05$) (Table 2). A significant increase in ferritin, and Hct, and a significant decrease in CRP, TIBC and WBC were observed in the second group ($p<0.05$) (Table 2).

Significant decrease was determined on the dietary intakes of some nutrients such as energy, total fat, saturated fatty acids and carbohydrates in both study groups. Dietary intakes of vitamin C, fiber, iron, and calcium were increased ($p<0.05$) (Table 3).

Discussion

Obesity and ID are two of the most common nutritional disorders in the worldwide. In the National Nutrition and Health Survey of Turkey, obesity was

Table 1. Changes in anthropometric measurements in women participating in a weight loss intervention

Anthropometric variables	**Hemoglobin (g/dL)**					
	Hb<12 g/dL (n=46)			**Hb≥12 g/dL (n=101)**		
	$\bar{x}$	**SD**	**p***	$\bar{x}$	**SD**	**p***
Weight (kg)			0.000*			0.000*
Before	84.9	18.1		85.0	17.0	
After	76.3	16.8		75.9	16.1	
BMI (kg/m²)			0.000*			0.000*
Before	32.3	6.5		33.2	6.3	
After	29.0	6.0		29.6	5.9	
Fat (%)			0.000**			0.000**
Before	37.7	5.7		39.2	5.6	
After	31.6	6.9		32.5	7.2	
Fat mass (kg)			0.000*			0.000*
Before	32.9	12.5		34.2	11.7	
After	25.1	11.6		25.7	11.1	
Fat free mass (FFM) (kg)			0.002*			0.002*
Before	52.0	6.2		50.8	5.8	
After	51.2	5.9		50.3	5.7	
Total body water (kg)			0.000*			0.000*
Before	38.0	4.5		37.2	4.2	
After	37.4	4.4		36.7	4.2	

**Wilcoxon signed rank test, p<0.05, **Paired Samples t test, p<0.05*

Table 2. Changes in biochemical measurements in women participating in a weight loss intervention

Biochemical parameters	Hemoglobin (g/dL)					
	Hb<12 g/dL (n=46)			Hb≥12 g/dL (n=101)		
	$\bar{x}$	SD	p*	$\bar{x}$	SD	p*
Iron (mcg/dL)			0.005*			0.187*
Before	44.3	20.9		75.7	35.1	
After	66.8	60.3		78.0	31.9	
CRP (mg/dL)			0.000*			0.001*
Before	0.6	0.4		0.6	0.6	
After	0.4	0.2		0.4	0.4	
Ferritin (ng/mL)			0.000*			0.000*
Before	6.8	6.0		17.4	12.6	
After	9.7	6.2		19.9	13.9	
Transferrin saturation (%)			0.01*			0.089*
Before	10.3	5.2		20.6	10.8	
After	16.6	14.2		21.5	9.5	
TIBC (mg/dL)			0.002**			0.005**
Before	436.9	61.2		379.3	51.2	
After	407.0	56.6		371.5	53.1	
WBC (mm^3)			0.267			0.02**
Before	7.4	2.3		7.2	1.8	
After	7.1	1.8		6.9	1.4	
Hemoglobin (g/dL)			0.000**			0.108**
Before	11.0	0.8		13.2	0.7	
After	12.1	1.2		13.3	0.8	
HCT (g/dL)			0.000**			0.016**
Before	34.3	2.5		39.8	1.9	
After	37.5	3.3		40.2	2.2	
MCV (g/dL)			0.000**			0.112**
Before	75.6	6.2		83.4	4.5	
After	79.2	6.4		83.8	4.9	

**Wilcoxon signed rank test, p<0.05, **Paired Samples t test; p<0.05, p>0.05, TIBC: Total iron binding capacity*

indicated as an important public health problem for Turkey. In adults, the prevalence of obesity and being overweight was reported as 30.3% and 34.6%, respectively (11). The results of various studies which were conducted in Turkey, shows that approximately 50% of lactating women were anemic (11).

The results of this intervention study revealed that iron status was improved by weight reduction in the both groups. Amato and colleagues, found an increased iron absorption among obese children who underwent a weight loss program although the iron measures did not significantly improve (6). Gong and colleagues, also indicated a relation between weight reduction and improved iron status, and inflammation. The improvement on inflammatory markers during weight reduction was indirectly associated with improved iron status (12).

In this study, Hb, an indicator of IDA, increased in the first group. Although, not statistically significant, Hb increased during the intervention period in the second group. Serum ferritin levels, which represent total iron stores were higher at the end of study compared to baseline in both groups (13). Contrary to this present study, Gong and colleagues reported that serum ferritin levels were lower at follow-up than baseline (12). Increased serum ferritin levels is an indicator

Table 3. Changes in dietary intakes in women participating in a weight loss intervention

	Hemoglobin (g/dL)					
Energy and Nutrients	**Hb<12 g/dL (n=46)**			**Hb≥12 g/dL (n=101)**		
	$\bar{x}$	**SD**	**p***	$\bar{x}$	**SD**	**p***
Energy (kcal/day)			0.000*			0.000*
Before	2020.6	291.0		2132.0	246.2	
After	1670.8	150.1		1709.7	122.2	
Protein (g)			0.000**			0.000**
Before	67.3	13.4		69.3	9.9	
After	76.6	11.7		75.6	10.3	
Animal Protein (g)			0.000*			0.000*
Before	33.9	11.7		32.3	10.2	
After	43.4	13.0		41.4	11.9	
Fat (g)			0.000*			0.000*
Before	80.0	17.9		83.8	16.6	
After	49.4	10.0		50.8	9.8	
Carbohydrate (g)			0.000*			0.000**
Before	251.4	43.5		266.8	38.5	
After	222.7	21.8		229.3	18.7	
Fiber (g)			0.000*			0.000**
Before	26.5	5.4		27.5	6.9	
After	31.0	4.9		29.8	5.3	*
Vitamin C (mg)			0.000**			0.000**
Before	192.5	80.7		175.8	65.2	
After	252.0	43.1		238.9	33.0	
Calcium (mg)			0.000**			0.000**
Before	792.1	225.0		852.1	204.9	
After	1007.2	158.8		1019.4	141.4	
Iron (mg)			0.000**			0.000*
Before	13.8	2.7		14.3	3.1	
After	17.1	1.9		17.2	1.8	

** Wilcoxon signed rank test, $p<0.05$, **Paired Samples t test; $p<0.05$*

of inflammation which is observed on obese children by Halle and colleagues (14). Increased serum ferritin levels are also positively associated with the obesity in adults (15). Ferritin may not be a good indicator of the ID in overweight and obese patients. In our study, ferritin levels were not high as an inflammatory marker. Further studies are needed to explain this result.

CRP, the classic acute-phase protein, is an exquisitely sensitive objective marker of inflammation, tissue damage, and infection (16). Heilbronn and colleagues reported elevated levels of CRP in obese, which declined during weight loss of 7 kg during a very low calorie diet (17). Tchernof and colleagues indicated a significant decrease in blood CRP which was shown in 25 obese, postmenopausal women participated in a weight loss program with a reduction of 14 kg of body weight (18). Contrary to previous reports, in a study of 21 obese women, no significant decrease in CRP was observed after weight loss of 3 weeks possibly due to the small number of subjects and a relatively modest weight loss of 3 kg (19). In this study, at baseline, CRP levels of groups were similar. The results of this study showed that plasma CRP levels decreased in both groups after 3 months intervention program possibly due to weight loss.

WBC count, as one of the major components of inflammatory process, plays an important role in pathogenesis of insulin resistance and cardiovascular disease. Elevated white blood cell counts (WBC) showed strong relations with obesity (20). In this intervention study, weight loss was resulted in decreased levels of WBC. Loss of ≥10% body weight was related to improvement on iron profile. An explanation for the relation between reduced BMI and improvement in iron status would be the improvement of inflammation (21,22). CRP and WBC levels were lower at the end of study, which suggests a reduction of inflammation.

Vitamin C impacts the absorption of dietary non-heme iron. There are finding suggested that vitamin C aids the absorption of non-heme iron through making it more soluble and breaks it down to the ferrous state (23).

Calcium can inhibit iron absorption, regardless of whether it is given as Ca salts or in dairy products. Increased Ca intake is recommended for children and women at the populations with high risk of ID. However, a through review of studies on humans in which Ca intake was substantially increased for long periods shows no changes in hematological measures or indicators of iron status. Thus, the inhibitory effect might occur in short term and also there might be compensatory mechanisms (24). Moreover, studies suggests that calcium-rich diets not only reduce the risk of cardiovascular disease but also play a direct role in the prevention and treatment of obesity (25). Fiber intake is inversely associated with body weight and body fat. Many mechanisms have been suggested for how dietary fiber aids in weight management, including promoting satiation, decreasing absorption of macronutrients, and altering secretion of gut hormones (26). After the intervention, dietary intake of energy, total fat, saturated fatty acids and carbohydrate decreased compared to the baseline. Dietary vitamin C, fiber, iron, and calcium intake increased compared to the baseline. Higher dietary intake of vitamin C and iron compared to the baseline would be caused a positive effect on iron profiles.

When muscle is broken down during exercise, myoglobin is released suggesting that higher levels of exercise could result in more bioavailable iron (27). In this study, women were suggested to walk 45 minutes for 3 days. Therefore, there is a possibility of increased physical activity levels to cause reduction of inflammation.

In summary, loss of ≥10% body weight caused improved the inflammatory and iron profile associated with decreased adiposity.

In this study, there are some limitations. Physical activity level was not recorded at baseline or at the end of study. Inflammation were only assessed by levels of blood CRP. Measurement of pro-inflammatory cytokines (TNF-alpha, interleukin-6, interleukin-8) would be useful parameters to determine inflammation in the further studies.

Acknowledgements

We thank the physicians who devoted their time to the data collection and participating women to the study.

All authors contributed to the study design. In addition, GK and GP collaborated on the first draft of the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by Hacettepe University Scientific Research Projects Unit Ph.D. Thesis Grant (012DO6401002).

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