

# Effect of Energy-Restricted Diet in Combination with Calcium Supplement or Low-Fat Milk on Iron Status of Overweight or Obese Premenopausal Women

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## Abstract

**Background:** Although it is assumed that calcium has beneficial effects on weight loss, the interaction of calcium and iron would be a major concern. We did this study to investigate the effects of calcium and low-fat milk on serum ferritin in overweight or obese premenopausal women.

**Methods:** Sixty-four healthy overweight or obese premenopausal women recruited in Shahid Beheshti University of Medical Sciences clinics participated in this clinical trial. Participants were randomly allocated to one of the following dietary regimens for 8 weeks: 1) a control diet providing a 500 kcal/day deficit, with 500-600mg/day dietary calcium; 2) a calcium-supplemented diet identical to the control diet with 800mg/day calcium carbonate 3) a milk diet providing a 500kcal/day deficit and containing three servings of low fat milk. Serum ferritin and anthropometric indices were measured at baseline and after 8 weeks. Primary outcome measure was serum ferritin level.

**Results:** Mean $\pm$ SD of baseline values for age, body mass index (BMI), waist circumference (WC), and serum ferritin were  $37.44\pm9.46$  (year),  $30.79\pm3.63$  ( $\text{kg}/\text{m}^2$ ),  $88.04\pm8.90$  (cm), and  $59.20\pm47.8$  ( $\mu\text{g}/\text{l}$ ), respectively. There were no significant differences in baseline age, BMI, WC, and serum ferritin among 3 groups. Mean values of serum ferritin reductions were  $0.26\pm20.36$ ,  $14.59\pm17.07$  and  $6.57\pm25.93$  ( $\mu\text{g}/\text{l}$ ) in control, calcium, and milk groups, respectively. Reduction in serum ferritin was only significant in the calcium group ( $P=0.003$ ). Serum ferritin reductions were not significantly different among the 3 groups ( $P=0.260$ ).

**Conclusion:** An energy-restricted diet in combination with calcium supplement or low-fat milk does not induce any additional adverse effect on iron status, compared to an energy-restricted diet alone.

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## Introduction

Calcium and iron status are two major public health concerns specifically in women.<sup>1</sup> Iron deficiency anemia

is a widespread condition worldwide,<sup>2</sup> with an estimated prevalence of about 10.3% in the developed and 42.3% in developing countries.<sup>3</sup> It can lead to adverse outcomes, such as fatigue, depression, and reduced work capacity.<sup>4</sup>

Iron status is affected by a variety of factors such as certain physiologic states, the degree of gastric acidity, and consumption of iron absorption enhancers and inhibitors including phytate, oxalate, and tannins.<sup>5</sup> It is suggested that consumption of an adequate amount of calcium enhances iron absorption via removing phosphate, oxalate, and phytate.<sup>5</sup> However, some professionals believe that calcium intake may have an adverse effect on iron absorption.<sup>6-8</sup> Halburg et al. found a negative dose response correlation between iron absorption and calcium intake from 40mg to 300mg.<sup>9</sup> In contrast, Cook et al. showed that consumption of 300mg or 600mg calcium has no effect on iron absorption.<sup>10</sup> Also, Gait et al. reported that taking less than 1000mg calcium causes no reduction in iron absorption.<sup>11</sup>

On the other hand, calcium is essential for bone health and its inadequate intake may lead to abnormal bone mass and osteoporosis.<sup>12</sup> Also, it is suggested that **calcium** and dairy products may have an effect on weight regulation.<sup>13-15</sup> Moreover, several studies have shown an independent association between higher dairy consumption and a more favorable lipid profile,<sup>16</sup> lower risks of hypertension,<sup>17</sup> insulin resistance, and type 2 diabetes.<sup>18-21</sup>

## Objectives

Given the concern about the interaction between calcium and iron, which are both of great nutritional importance in women, and regarding the fact that low-calorie diets are also deficient in some micronutrients specifically iron, we aimed to assess the effects of an energy-restricted diet in combination with calcium supplement or low-fat milk on iron status of overweight or obese premenopausal women.

## Material and Methods

### Study Sample

The sample size was computed on the basis of having an effect size equal to 0.55. A minimum of 20 participants per group was calculated with a power of 80% and a type I error of 5%. Estimating 20% drop-out rate, we recruited 25 persons in each group.

Seventy five eligible healthy overweight or obese, and otherwise healthy premenopausal women from Shahid Beheshti University of Medical Sciences clinics volunteered to participate in this randomized controlled trial, and 64 women completed the study. Eleven (14.6%) subjects dropped out for various reasons, including not consuming calcium supplement and unwillingness to continue participation (Figure 1). Eligible participants required to have the following conditions: a body mass index (BMI) of >25 kg/m<sup>2</sup>, ages between 20-50 years, and ability to understand

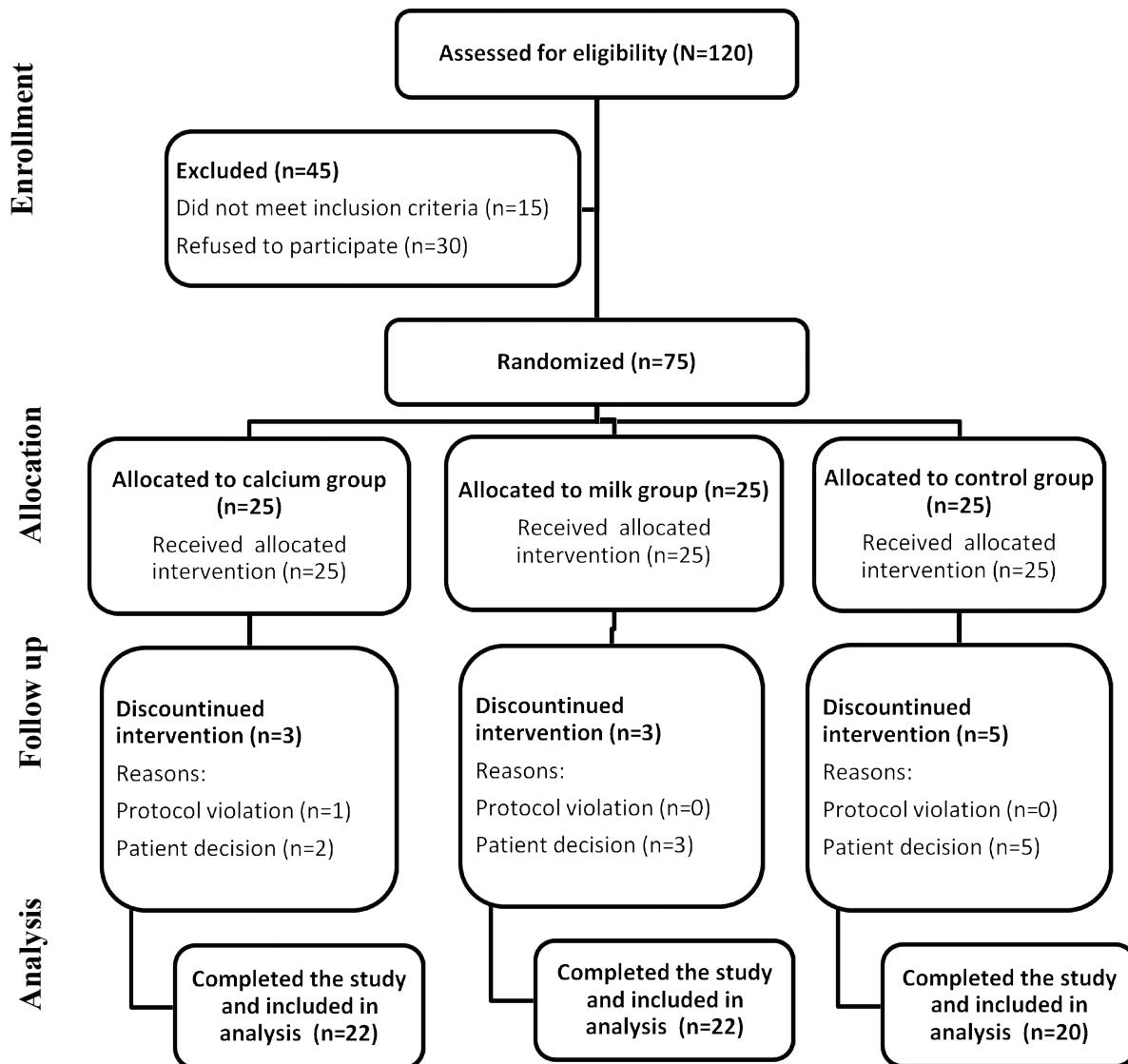
the study protocol and provide written informed consent. Exclusion criteria included: taking any medication or supplement which might affect weight loss or metabolism of iron, calcium, and vitamin D; a body weight change of >3kg during the last 2 months; pregnancy, lactation, or menopause; lactose intolerance or allergy to milk; diagnosis of diabetes mellitus, hypertension, or thyroid, kidney, and coronary artery diseases; participating in any other studies within the last 6 months before screening; and lack of adherence to the research protocol.

Each potential participant was informed of the possible risks and benefits associated with this study and provided written signed consent. The study was approved by the ethics committee of National Nutrition and Food Technology Research Institute, and the research protocol was registered in IRCT with the ID of IRCT201304213080N1.

### Study Interventions

This study was carried out from October 2009 to March 2010. The subjects were studied for a 2-week run-in period for baseline dietary and physical activity assessment, and then using balance block method in a 1:1:1 ratio were randomly allocated the subjects to one of the following dietary regimens for 8 weeks: 1) a control diet providing a 500 kcal/day deficit, with 500-600 mg/day dietary calcium; 2) a calcium-supplemented diet identical to the control diet with 800 mg/day of calcium (as calcium carbonate); 3) a milk diet providing a 500 kcal/day deficit and containing three servings (220ml) of low fat milk (1.5%). The total calcium content of the milk diet was between 1200-1300 mg/day. Daily energy requirements were calculated using Harris-Benedict equation.<sup>22</sup> After multiplying the activity factor, a meal plan based on a 500 kcal/day deficit from estimated energy requirements was given to each individual.<sup>23</sup> Macronutrient distribution of diets for 3 groups was as follows: 55% carbohydrate, 18% protein, and 27% fat. The participants were provided with milk and calcium supplements every 2 weeks. Calcium supplements were manufactured by Iran Daru Company. Each caplet contained 400 mg of calcium as calcium carbonate. Sterilized milk containing 1.5% fat was bought from Pegah Company in Tetra-pack form. Compliance to calcium supplement was assessed by counting the remainder of caplets.

Before starting the study, the participants were asked about their age, marital status, health status, and medications. At baseline and at two-week intervals, the participants' weight, height, and waist circumference were measured. Physical activity and 24h-dietary records (2 week days and 1 weekend day) were also taken. Weight was measured in light clothing using a digital scale to the nearest 100 gram. We measured the

**Figure 1:** Participant's flow diagram throughout the study

height bare foot by using a stadiometer to the nearest 0.1 cm. BMI was then calculated as weight (kg)/height ( $m^2$ ). Waist circumference was measured using a non-stretch tape measure on the narrowest part of the waist to the nearest 0.1 cm. Physical activity was estimated as MET.h/day. Also, at baseline and after 8 weeks, 5cc blood sample was obtained after 10h fasting in the morning. Blood samples were centrifuged (500 rpm, 15 min) and the sera were frozen at -80°C for later analysis. Primary outcome measure was serum ferritin level, which was measured by ELISA method (Diagnostic Biochem, Ontario, Canada) at baseline and after 8 weeks.

#### Statistical Analysis

Dietary records were analyzed using Nutritionist IV software (First Data bank, Hearst Corp, SanBruno, CA, USA). Statistical analyses were performed by using SPSS software, version 16 (IBM, Armonk, NY, USA). One way ANOVA was used to compare

the continuous characteristics of the subjects in the 3 groups at baseline, and also to compare the mean changes of serum ferritin among 3 groups. ANCOVA was used to remove the effects of baseline serum ferritin values and changes of anthropometric indices on serum ferritin changes after the intervention. The effect of intervention on each group was assessed using paired t-test. A P<0.05 was considered significant.

#### Results

Of the 75 women meeting the general eligibility criteria, 11 dropped out before completing the weight-loss period (5, 3, and 3 persons in control, calcium, and milk groups, respectively) (Figure 1). However, no adverse effect of taking the supplements or milk consumption was reported by the participants.

Baseline characteristics of the participants are presented in Table 1. Mean±SDof baseline values for the participant's age, BMI, WC, and serum

**Table 1:** Comparison of baseline characteristics of participants in the three groups

Variables	Control (n=20)	Calcium (n=22)	Milk (n=22)	P*
Age (year)	38.25±9.49	35.77±8.70	38.27±10.43	0.61
Weight (kg)	76.78±9.6	78.16±11.43	76.24±10.57	0.78
WC (cm)	90.5±2.02	90.77±1.93	86.84±1.93	0.09
BMI (kg/m <sup>2</sup> )	30.78±3.13	31.54±4.12	30.01±3.55	0.39
Energy (kcal/day)	1839.35±169.54	1870.77±201.79	1937.26±177.79	0.25
Calcium (mg/day)	512.85±172.71	532.29±149.77	484.58±131.07	0.64
Physical activity (MET.h/day)	35.48±4.37	33.90±3.46	33.17±3.04	0.12
Serum ferritin (μg/l)	60.90±46.75	59.14±47.28	57.66±43.52	0.69

All values are mean±standard deviation. BMI, body mass index; MET, metabolic equivalent; WC, waist circumference, \*One way ANOVA

ferritin were 37.44±9.46 (year), 30.79±3.63 (kg/m<sup>2</sup>), 88.04±8.90 (cm), and 59.20±47.8 (μg/l), respectively. There were no significant differences in the subject's age, anthropometric indices, dietary intakes (energy, iron, and calcium), physical activity, and serum ferritin among the 3 groups at baseline.

As shown in Table 2, there were no significant differences in energy, protein, fiber, and iron intakes among the 3 groups during the study.

Table 3 shows that under energy-restricted diet, the participants in all groups had a significant weight, BMI, and WC reduction (P<0.001 for all). However, these reductions were not significantly different among the 3 groups for weight and BMI. Differences of serum ferritin changes among the 3 groups were not statistically significant (P=0.260), and also remained non-significant after adjusting for baseline values of serum ferritin and changes in anthropometric indices including weight, WC, and BMI, using ANCOVA (P=0.330). Serum ferritin was only reduced significantly in the calcium group (P=0.003).

## Discussion

Results of our study indicated that after 8 weeks of intervention, serum ferritin was reduced significantly in the calcium supplemented group. Although serum ferritin reduction was observed in the controls and the milk group, the difference was not statistically significant. Also, there were no significant differences among the serum ferritin reductions of the three groups. It seems that consumption of 3 serving of milk or 800mg of calcium supplement did not cause any extra reduction in serum ferritin compared to energy limited diet alone.

In agreement with our results, Grinder-Pedersen and colleagues reported that non-heme iron absorption did not differ significantly among four different 3-day diets including a basic diet (BD) with a low content of calcium (224 mg Ca/day), a BD with a glass of milk served at each meal (826 mg Ca/day), a BD with calcium lactate (802 mg Ca/day), and a BD with a milk mineral isolate containing calcium (801 mg Ca/day).<sup>6</sup> Also, Abrams and colleagues found that consumption of calcium fortified cereals (containing 159 mg of

**Table 2:** Participants' Intake of energy, protein, fiber, iron, and calcium during the study period

Variables	Control (n=20)	Calcium (n=22)	Milk (n=22)	P*
Energy (kcal/day)	1221.21±153.73	1239.60±180.09	1297.89±137.83	0.25
Protein (% of calorie intake)	17.63±1.34	17.40±2.78	17.59±2.21	0.90
Fiber (g/day)	14.34±3.76	14.37±4.11	13.77±2.35	0.80
Iron (mg/day)	7.64±1.81	7.62±1.99	7.40±1.14	0.95
Calcium (mg/day)	495.46±163.87 <sup>ab</sup>	1320.53±219.36 <sup>a</sup>	1302.00±107.56 <sup>b</sup>	<0.001

All values are mean±standard deviation. \*One way ANOVA, Same letters shows significant differences by Tukey post hoc test (a and b P<0.001)

**Table 3:** Changes in anthropometric indices and serum ferritin of participants after the intervention

Variables	Control (n=20)		Calcium (n=22)		Milk (n=22)		P*	P**
	Before	After	Before	After	Before	After		
Weight (kg)	76.78±9.60	73.92±9.76	<0.001	78.16±11.43	74.27±9.91	<0.001	76.24±10.57	71.83±9.76
BMI (kg/m <sup>2</sup> )	30.78±3.13	29.64±3.12	<0.001	31.54±4.12	29.99±3.52	<0.001	30.01±3.55	28.29±3.51
WC (cm)	90.5±2.02	86.55±7.78	<0.001	90.77±1.93	85.61±8.03	<0.001	86.84±1.93	80.56±7.88
Serum ferritin (μg/l)	60.90±46.75	60.70±38.64	0.980	59.14±47.28	58.59±71.37	0.003	57.66±43.52	48.90±38.20
							0.270	0.264†

All values are mean±standard deviation. BMI, body mass index; WC, waist circumference, \*Paired T-test†, \*\*One way ANOVA, †After adjusting for baseline value of serum ferritin and changes of weight, WC, and BMI using ANCOVA, the p-value remained non-significant (P=0.260)

calcium per serving) in 4 to 6 year old children for 14 days did not interfere with iron absorption.<sup>24</sup> Damms-Machado et al. carried out a pilot multidisciplinary weight loss program, in which 32 subjects were followed up for micronutrient status before and after a three-month formula diet. The average intake of iron during the study period was  $12.9 \pm 0.36$  mg/day in women and  $14.7 \pm 0.79$  mg/day in men. In spite of receiving as much as RDA, they observed more subjects with iron, vitamin C, selenium, and zinc deficiency after a three-month period of formula diet intervention, and concluded that DRI-covering low-calorie formula diet cannot meet the nutritional needs of obese individuals.<sup>25</sup> With regard to Damms-Machado and coworkers results, iron intake of less than DRI is a possible explanation for worsening of iron status after the intervention. Also, it is suggested that the level of ferritin as a positive acute-phase protein is elevated in obese people and decreased following weight loss.<sup>4,26</sup>

On the other hand, Benkhedda and co-workers reported that intake of 500mg/day calcium carbonate in women with marginal iron status results in 4.8% to 10.2% reduction in iron absorption from a single meal.<sup>1</sup> Using radioactive tracer, Gait et al. investigated the effect of 200-800mg/day calcium chloride on the absorption of 5mg heme iron, and also the effects of calcium doses between 200 and 1500 mg/day on the absorption of 5mg non-heme iron. They observed reductions in the absorption of heme and non-heme iron with calcium doses of 800mg/day and  $\geq 1000$  mg/day, respectively; however, they did not find any adverse effect with taking smaller doses.<sup>11</sup> Putting it all together, it seems that taking less than 800mg/day of supplemental calcium does not endanger iron status.

Although the mechanism by which calcium reduces iron absorption is not well understood, it is assumed that calcium inhibits the serosal transfer of iron.<sup>27</sup> However, it is suggested that because of adaptation to a high calcium diet, the effect of increased calcium intake on iron absorption may not persist in the long-term.<sup>24</sup>

Despite similar calcium intake, we found a greater but non-significant reduction in serum ferritin in the calcium group, compared to the milk group. This finding might be explained by the fact that casein and its derived peptides in the dairy products have potent mineral binding properties which could lead to increased iron absorption.<sup>28</sup>

To the best of our knowledge, this is the first study which assessed the effects of milk consumption or Ca supplementation on iron status for as long as 2 months. But as the main limitation of our study, we can refer to the fact that the present study was the result of a supplementary analysis of our original data on the assessment of the effect of milk or calcium

consumption on weight loss.

In conclusion, it seems that consumption of calcium supplement as much as 800mg/day or 3 servings of milk in combination with energy-restricted diet does not induce any additional adverse effect on iron status, compared to an energy-restricted diet alone. However, our findings suggest that calcium supplementation must be done with caution, and to avoid iron depletion in individuals on weight reducing diets, paying more attention to iron intake is necessary.

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**Conflict of Interest:** None declared.

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