Original Article

Impact of diet and weight loss on iron and zinc status in overweight and obese young women

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Young overweight women are at risk of iron and zinc deficiency. This study assessed iron, zinc and inflammatory status during a 12-month weight loss trial in young women (18-25 y; BMI ≥27.5 kg/m^2) randomised to a higher-protein (HP: 32% protein; 12.2 mg/day iron; 11.7 mg/day zinc) or lower-protein (LP: 20%; 9.9 mg/day; 7.6 mg/day respectively) diet with contrasting haem iron and zinc content. In completers (HP: *n*=21; LP: *n*=15), HP participants showed higher median ferritin (52.0 vs 39.0 µg/L; *p*=0.021) and lower median soluble transferrin receptor-ferritin index (sTfR-F; 0.89 vs 1.05; *p*=0.024) although concentrations remained within normal range for both diets. Median C-reactive protein (CRP; HP: 3.54; LP: 4.63 mg/L) and hepcidin (HP: 5.70; LP: 8.25 ng/mL) were not elevated at baseline, and no longitudinal between-diet differences were observed for zinc and CRP. Compared to those with <5% weight loss, HP participants losing ≥10% weight showed lower median sTfR-F (0.76 vs 1.03; *p*=0.019) at six months. Impact of ≥10% weight loss on iron was more apparent in LP participants who exhibited greater mean serum iron (20.0 vs 13.5 µmol/L; *p*=0.002), transferrin saturation (29.8% vs 19.4%; *p*=0.001) and lower sTfR (1.24 vs 1.92 mg/L; *p*=0.034) at 12 months. Results show normal iron and zinc status can be maintained during 12 months of energy restriction. In the absence of elevated baseline inflammation and hepcidin, a more favourable iron profile in those with ≥10% weight loss may reflect stronger compliance or the potential influence of iron regulatory mechanisms unrelated to inflammatory hepcidin reduction.

Key Words: obesity, weight loss, young adult, iron, zinc

INTRODUCTION

Young women are at an increased risk of weight gain and obesity.[1] At this life stage, factors such as moving away from home, cohabitation and pregnancy are all associated with weight gain.[2,3] In addition to the deleterious effects on metabolic and reproductive health,[4,5] obesity is also linked with abnormal micronutrient metabolism, particularly for calcium, iron, zinc, vitamins A, D and folic acid.[6-8] These micronutrient disturbances may contribute to the aetiology of obesity, as supported by studies showing leptin suppression in zinc deficiency,[6,9] or arise from excess adiposity such as the hypoferraemia of obesity.[10]

Obesity-related alterations to micronutrient concentration are of particular concern in young women as: 1) requirement for nutrients such as iron are higher in this population;[3] 2) obesity-related inflammation may contribute to reduced micronutrient status;[11,12] and 3) unhealthy eating habits can compromise nutrient adequacy.[13] Dietary iron intake, particularly haem iron, is often reported to be low in young women,[14-16] and reduced food consumption even in nutritionally balanced diets can limit micronutrient intake.[3] As iron and zinc have been reported as limiting nutrients in energy-restricted diets for young women,[3] requirement for these nutrients may be more easily met using animal-based, higher-protein meal plans that are nutrient dense and provide iron and zinc in the most bioavailable form.[3]

Current literature examining micronutrient (particularly iron and zinc) alteration during medium to longer term (minimum six months) diet-induced weight loss is limited. Many studies focus on bariatric surgery-induced weight loss which can affect nutrient absorption.[17] Some report favourable changes to iron, zinc and inflammatory status,[18-21] although the impact of different degrees of weight loss on these biomarkers is unknown. With reduced iron, zinc and elevated inflammatory status all correlated to increasing BMI,[6] it is reasonable to assume that a larger reduction in excess weight will result in greater improvement of iron, zinc and inflammatory status.

The objective of this study was to compare the effect of two energy-restricted diets with contrasting protein, haem

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iron and zinc content as well as differing degrees of weight loss (<5% vs ≥10%) induced by these diets on iron, zinc and inflammatory status of young overweight and obese women. We hypothesise that participants who lose ≥10% of initial weight with either diet will have superior iron and zinc status and a lower level of inflammation than those on the same diet but who were less successful (<5%) with weight loss. Additionally, amongst the successful participants, those who lose ≥10% of weight via the higher-protein, haem iron and zinc diet would show a more favourable iron and zinc profile to participants on a diet containing lower protein, haem iron and zinc content.

MATERIALS AND METHODS

Study design

This study examined iron, zinc and inflammatory status in young overweight women who completed a 12-month single-blind randomised controlled trial comparing the efficacy of two iso-energetically restricted diets (5600 kJ/day) differing in macronutrient, haem iron and zinc content (HP: higher-protein, haem iron and zinc diet; LP: lower-protein, haem iron and zinc diet) on weight loss.[22] All participants were provided with an identical behaviour modification programme and a standard exercise prescription based on national activity guidelines.[23,24] Participants attended counselling sessions weekly up to three months, fortnightly from three to six months and monthly from six to 12 months.

Participants

Healthy women aged 18-25 years with a measured BMI ≥27.5 kg/m^2 were recruited. Exclusion criteria were applied to minimise confounding on weight loss outcomes.[22] Since iron and zinc status were important biochemical outcomes of interest and animal protein was included in both diets, volunteers were ineligible if they had any haematological disorders, were anaemic (haemoglobin (Hb) <120 g/L) or vegetarian. As iron deficiency without anaemia (serum ferritin <15.0 μg/L) is prevalent in this population, iron deficient volunteers were advised to take the same ferrous fumarate supplement (10 mg elemental iron per day, five days a week) for three months and randomised upon normalisation of iron status and discontinuation of iron supplementation. Self-reported use of contraceptive medication was recorded due to its confounding effect on iron and zinc status.[25] Recruited participants were required to cease all dietary supplements and blood donation for the entire duration of the trial. Volunteers reporting recent (week prior) acute infection had venepuncture (at baseline, six and 12 months) rescheduled to avoid infection-related inflammation.

Dietary intervention, compliance and estimated dietary iron and zinc intake

The HP diet provided 32% protein; 41% carbohydrate; 25% fat; 12.2 mg iron; and 11.7 mg zinc (8.20 mg from animal sources) per day, whereas the LP diet provided 20% protein; 58% carbohydrate; 21% fat; 9.90 mg iron; and 7.60 mg zinc (3.60 mg from animal sources). Haem iron, estimated as 40% of total iron from meat, poultry, and fish,[26,27] was calculated at 1.90 and 0.40 mg/day for the HP and LP diets respectively. Both diet plans met the Australian estimated average requirement (EAR) for iron and zinc, but not the recommended dietary intake (RDI) for iron (HP: 68%; LP: 55%).[28] The LP diet also did not meet the RDI for zinc (95%). Further details on the dietary prescriptions have been published elsewhere.[22]

To monitor recent protein intake and compliance to the protein prescription, 24-h urine samples were collected for measurement of urea/creatinine ratio (UCR).[29] As studies have reported the need for at least 12 days of dietary data to estimate iron intake confidently,[30] average iron and zinc intake were estimated using three-day food records collected at the end of the first, second, third, sixth and twelfth months- totalling 15 days for trial completers. To maximise accuracy, training on how to record food intake using household measures was provided by a dietitian. Kitchen scales were also given to each participant. When required, recorded food portions were verified with the assistance of a visual aid.[31] Nutrient analysis was performed using FoodWorks Version 6.0.25175 (Xyris Software, Brisbane, Australia).

Anthropometry

Height (nearest 0.1 cm) and weight (nearest 0.1 kg; measured at every visit) were recorded using a wall-mounted stadiometer (Hyssna Limfog AB, Hyssna, Sweden) and digital platform scale (Teraoka Seiko, Tokyo, Japan) respectively. Waist circumference was measured at baseline, three, six and 12 months according to international guidelines.[32]

Biochemical analysis

Fasting morning venous blood samples were collected at baseline, six and 12 months. Hb, serum iron, transferrin saturation, serum ferritin and plasma zinc were analysed at a nationally accredited commercial diagnostic laboratory. Samples for zinc analysis were collected in trace element-free tubes in accordance with the International Zinc Nutrition Consultative Group (iZiNCG) recommendations and analysed using inductively coupled plasma mass spectroscopy (Perkin-Elmer, Waltham, USA) at the same commercial diagnostic laboratory.[33] Reference ranges were 120-165 g/L for Hb; 10.0-30.0 μmol/L for serum iron; 12.0-45.0% for transferrin saturation; 15.0-165 μg/L for ferritin; and 10.0-20.0 μmol/L for zinc. Plasma was stored for analysis of soluble transferrin receptor (sTfR) and C-reactive protein (CRP) using commercial ELISA kits (R&D Systems, Minneapolis, USA). Median inter-assay coefficients of variation reported in the kits were 5.70% and 6.60% for sTfR and CRP respectively. Reference ranges were 0.740-2.39 mg/L for sTfR and 0.110-4.52 mg/L for CRP. sTfR results were converted from nmol/L to mg/L.[34] Clinically elevated CRP was defined as >10.0 mg/L.[35] The sTfR-ferritin index (sTfR-F), described as a useful indicator of iron status in inflammation was calculated.[36] Plasma hepcidin was measured only at baseline using an on-line extraction coupled to liquid chromatography-tandem MS method with the Xevo TQ MS (Waters Corporation, Milford, USA).[37] Inter-assay accuracy was 95.0% with an 8.20% coefficient of variation. Assay sensitivity was 2.00 ng/mL with values below

the detectable range defined as 1.00 ng/mL. A reference range of 1.92-32.4 ng/mL was used.[38, 39]

Ethics

This study was registered with the Australian New Zealand Clinical Trials Registry (ID: ACTRN12609000307202). All procedures were in accordance with the ethical standards of the Sydney South West Area Health Service Ethics Review Committee and the Human Research Ethics Committee of The University of Sydney. Signed and informed consent was obtained from all participants.

Statistical analysis

Statistical analysis was performed using PASW Statistics 18 for Windows (IBM Corporation, Armonk, USA). As weight loss was the primary outcome of this trial, sample size was calculated on the basis of detecting a weight difference between the diet groups. Based on a significance level of 0.05 and 80% power, 28 participants were required in each diet to detect a 5 kg weight difference. With attrition estimated at approximately 20%,[40] a recruitment goal of 70 participants was established.

In this study, data were analysed only for participants who completed six and 12 months to determine true biochemical alterations associated with the dietary intervention which were not based on statistical assumptions used for treating missing data. Participants were categorised into non-responder (<5% loss of initial weight) and responder (≥10% loss of initial weight) groups within each diet to examine the influence of weight loss success on the biochemical markers. These cut-offs were selected as they have previously been used to reflect weight loss success,[41] and 10% weight reduction is generally associated with important improvements in chronic disease risk factors.[42] Categorising participants according to these cut-offs also allow for a clear distinction between the two degrees of weight loss.

Variables were assessed for normality with natural log transformations performed on the serum ferritin, sTfR-F, CRP and hepcidin variables. Baseline age, weight, BMI and the dietary compliance measures (UCR and dietary iron and zinc intake) were compared between the two diets using unpaired t-tests. Paired t-tests were used to assess anthropometric changes (from baseline) within each diet. Pearson's tests were used to correlate dietary iron and zinc intake against serum ferritin and plasma zinc concentration. Repeated measures ANOVA (adjusted for baseline BMI) was used to compare anthropometric changes between the diets. Biochemical differences between the diets and response groups at baseline and the longitudinal time points (where the baseline value of the dependent variable was included as an additional covariate) were assessed using ANCOVA. All ANCOVA analyses were adjusted for baseline BMI and contraceptive medication, whereas ANCOVA tests comparing serum iron, transferrin saturation, ferritin and zinc concentrations were also adjusted for lnCRP (to account for potential inflammatory differences). Significance was set to $p<0.05$, with data presented as percentages, mean±SD or median (range).

RESULTS

A total of 71 participants (HP: n=36; LP: n=35) were recruited to the trial with 44 (HP: n=24; LP: n=20) completing six and 36 (HP: n=21; LP: n=15) completing to 12 months. Reasons for high attrition in this trial have been described.[22] Results reported in this study refer to six and 12-month completers only. All baseline characteristics were similar between diets with the exception of higher BMI in HP participants (Table 1). Use of contraceptive medication was reported by 36%. At recruitment (prior to pre-trial iron supplementation), iron deficiency (ferritin <15.0 µg/L) was identified in six (17%; HP: n=4; LP: n=2) participants and marginally low plasma zinc (9.50 µmol/L; 5% below the lower normal limit) in one participant randomised to HP. All participants presented with baseline hepcidin concentration within normal range and clinically elevated CRP (>10.0 mg/L) was observed in five (HP: n=1; LP: n=4) participants. As baseline hepcidin was unremarkable indicating minimal likelihood of substantial hepcidin-mediated iron disturbances, measurement of this marker at six and 12 months was not pursued. Mean values for all biomarkers were within normal range throughout the trial.

Weight loss

Both diets independently led to significant weight loss at six (HP: 9.31±8.87%, $p<0.001$; LP: 5.08±5.99%; p=0.001) and 12 months (HP: 9.79±13.0%, p=0.003; LP: 4.56± 7.15%, p=0.027). Between the diets, weight loss in HP was approximately double than that of LP, although this was not statistically significant (Figure 1). Similar between-diet outcomes were observed for waist circumference at six (HP: -7.8±1.3; LP: -3.7±1.0 cm; p=0.30) and 12 months (HP: -7.9±1.8; LP: -2.4±0.8 cm; p=0.36). Other anthropometric outcomes have also been published.[22] At six months, 18 non-responders (HP: n=8; LP: n=10) lost <5% of initial weight while 13 responders (HP: n=10; LP: n=3) achieved losses of ≥10%. By 12 months, 27 of the 36 participants who completed the trial had lost <5% (HP: n=6; LP: n=8) or ≥10% (HP: n=9; LP: n=4) of weight. The distribution of responders and non-responders was not significantly different between the diets (six months: p=0.127; 12 months: p=0.153).

Dietary compliance and estimated iron and zinc intake

No significant UCR difference was observed between the diets at baseline (HP: 33.5±7.43; LP: 30.6±6.94; p=0.194). At six months, UCR was significantly higher on the HP diet (HP: 38.4±9.67; LP: 30.5±7.31; p=0.023) which was consistent with the protein prescription. However, this difference was no longer significant at 12 months (HP: 35.0±7.40; LP: 33.7± 5.49; p=0.547), indicating reduced compliance.

Estimated iron and zinc intake is presented in Table 2. As expected, iron and zinc intake in HP was significantly higher than the LP diet (all $p<0.001$) and was close to the dietary prescription for both groups. No significant differences in iron or zinc intake were observed between response groups in either diet (Table 3). All HP participants met the EAR for dietary iron and zinc while a number of participants on the LP diet did not (Table 2).

Table 1. Comparison of participant age, anthropometric and biochemical characteristics between diets at baseline

Baseline characteristic	6-month completers at baseline			12-month completers at baseline		
	HP diet (*n*=24)	LP diet (*n*=20)	*p* value	HP diet (*n*=21)	LP diet (*n*=15)	*p* value
Age (y)	22.4 ± 2.3	21.8 ± 2.2	0.339	22.4 ± 2.3	22.1 ± 2.1	0.678
Weight (kg)	95.8 ± 9.1	92.9 ± 11.7	0.368	96.2 ± 8.9	92.5 ± 11.6	0.291
Ethnicity (%)						
European	83.3	75.0		81.0	86.7	
Asian	4.17	10.0		4.76	6.67	
African	0.00	5.00		0.00	6.67	
South American	4.17	10.0		4.76	0.00	
Other	8.36	0.00		9.52	0.00	
BMI (kg/m^2)	34.3 ± 3.5	32.2 ± 3.6	0.063	34.6 ± 3.4	32.2 ± 3.6	0.047
Hb (g/L)	131 ± 8	130 ± 9	0.797	132 ± 8	130 ± 10	0.456
Serum iron (µmol/L)	13.5 ± 6.2	16.3 ± 7.2	0.344	14.0 ± 6.2	15.5 ± 6.2	0.749
Tsat (%)	19.5 ± 8.5	24.7 ± 12.2	0.147	20.5 ± 8.4	23.9 ± 11.4	0.393
Serum ferritin (µg/L)†	33.0 (89.0)	37.0 (185.0)	0.286	34.0 (80.0)	35.0 (82.0)	0.985
<15.0 µg/L (%)	16.7	10.0		9.52	13.3	
sTfR (mg/L)	1.56 ± 0.34	1.67 ± 0.61	0.445	1.55 ± 0.35	1.73 ± 0.66	0.356
sTfR-F index†	1.01 (1.52)	1.02 (1.60)	0.652	0.95 (1.50)	1.01 (1.42)	0.839
Hepcidin (ng/mL)†	5.70 (24.6)	8.25 (24.3)	0.123	5.80 (24.6)	7.40 (24.0)	0.298
Zinc (µmol/L)	14.0 ± 2.4	14.8 ± 1.9	0.216	14.0 ± 2.5	14.5 ± 1.8	0.381
<10.0 µmol/L (%)	4.17	0.00		4.76	0.00	
CRP (mg/L)†	3.55 (13.2)	4.63 (27.1)	0.690	3.51 (13.2)	4.75 (13.0)	0.626
>10.0 mg/L (%)	4.17	20.0		4.76	13.3	

Mean±SD or median (range). HP, higher-protein; LP, lower-protein; Tsat, transferrin saturation; sTfR, soluble transferrin receptor; sTfR-F, soluble transferrin receptor-ferritin index; CRP, C-reactive protein
Reference ranges – Hb: 115-165 g/L; serum iron: 10.0-30.0 µmol/L; Tsat: 12.0-45.0%; ferritin: 15.0-165 µg/L; sTfR: 0.74-2.39 mg/L; zinc: 10.0-20.0 µmol/L; CRP: 0.11-4.52 mg/L; hepcidin: 1.92-32.4 ng/mL
†Natural log transformation performed on the ferritin, sTfR-F, CRP and hepcidin variables

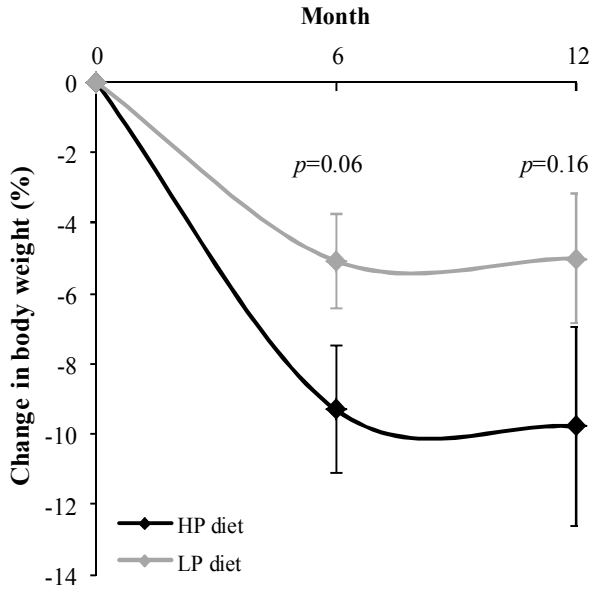

Figure 1. Percent loss of initial weight between the diets (mean±SE)

Pearson's tests revealed no significant correlation between iron intake and serum ferritin (12 months: r=0.109; *p*=0.547), although a positive trend for higher plasma zinc with greater zinc intake was observed (12 months: r=0.322; *p*=0.068).

Iron, zinc and inflammatory status between diets

Comparison of micronutrient and inflammatory markers between the diets is presented in Table 2. At 12 months, HP participants showed changes reflective of increased iron stores with significantly higher ferritin [HP: 52.0 (132) vs LP: 39.0 (65.0) µg/L; *p*=0.021] and lower sTfR-F [HP: 0.89 (1.04) vs LP: 1.05 (2.96); *p*=0.024]. In contrast, these markers remained stable in LP participants throughout the trial. Plasma zinc and CRP decreased from baseline in both diet groups, although no significant between-diet differences were observed. Use of contraceptive medication was a significant confounder of several iron markers including serum ferritin (*p*=0.032), sTfR (*p*=0.019) and sTfR-F (*p*=0.001) (results from 12-month data reported for brevity).

Of the six (HP: *n*=4; LP: *n*=2) participants identified as iron deficient at recruitment, three (HP: *n*=2; LP: *n*=1) were able to maintain normal ferritin following 12 months of intervention. Conversely, in those with normal baseline iron status, four participants (HP: *n*=2; LP: *n*=2) developed iron deficiency upon trial completion and had to be referred to their family physician for follow-up treatment. The number of HP participants presenting with low ferritin decreased from baseline to 12 months whereas the opposite was observed in the LP diet group (Tables 1 and 2). In the one HP participant who initially presented with low plasma zinc, zinc concentration was normalised after six months which was maintained at 12 months. At trial completion, low plasma zinc was observed in two participants (HP: *n*=1; HC: *n*=1).

Iron, zinc and inflammatory status between response groups

Table 3 shows the biochemical comparisons between diet responders (<5% weight loss) and non-responders (≥10% weight loss). Within each diet at six months, HP responders showed significantly lower sTfR-F [non-responders: 1.03 (0.92) vs responders: 0.76 (0.40); *p*=0.019] while LP responders exhibited a trend for higher ferritin [non-

Table 2. Comparison of biochemistry and micronutrient intake between the diets at six and 12 months

Biochemical or intake variable	6 months			12 months		
	HP diet (n=24)	LP diet (n=20)	*p* value	HP diet (n=21)	LP diet (n=15)	*p* value
Hb (g/L)	130 ± 11	128 ± 7	0.318	132 ± 12	126 ± 11	0.075
Serum iron (μmol/L)	14.3 ± 6.4	15.0 ± 4.5	0.564	15.6 ± 5.3	15.5 ± 8.8	0.242
Tsat (%)	21.9 ± 10.3	22.1 ± 7.4	0.410	23.7 ± 8.6	23.5 ± 14.4	0.244
Serum ferritin (μg/L)†	46.0 (107)	37.0 (141)	0.052	52.0 (132)	39.0 (65.0)	0.021
<15.0 μg/L (%)	12.5	5.00		9.52	20.0	
sTfR (mg/L)	1.50 ± 0.39	1.72 ± 0.69	0.335	1.46 ± 0.30	1.68 ± 0.71	0.145
sTfR-F index†	0.80 (1.58)	0.98 (2.55)	0.057	0.89 (1.04)	1.05 (2.96)	0.024
Zinc (μmol/L)	13.6 ± 1.7	13.2 ± 1.3	0.081	13.1 ± 2.3	12.1 ± 1.4	0.159
<10.0 μmol/l (%)	0.00	0.00		4.76	6.67	
CRP (mg/L)†	2.36 (14.9)	2.65 (21.8)	0.597	2.22 (25.8)	3.95 (12.0)	0.784
>10.0 mg/L (%)	12.5	20.0		14.3	6.67	
Iron intake (mg/day)	11.9 ± 2.7	8.3 ± 1.6	<0.001	11.4 ± 1.9	8.5 ± 1.5	<0.001
<EAR (%)	0.00	30.0		0.00	26.7	
<RDI (%)	95.8	95.0		90.5	93.3	
Zinc intake (mg/day)	12.3 ± 2.4	6.8 ± 1.4	<0.001	11.8 ± 1.7	7.2 ± 1.3	<0.001
<EAR (%)	0.00	40.0		0.00	26.7	
<RDI (%)	4.17	80.0		0.00	73.3	

Mean±SD or median (range). HP, higher-protein; LP, lower-protein; Tsat, transferrin saturation; sTfR, soluble transferrin receptor; sTfR-F, soluble transferrin receptor-ferritin index; CRP, C-reactive protein; EAR, estimated average requirement; RDI, recommended dietary intake

Reference ranges – Hb: 115-165 g/L; serum iron: 10.0-30.0 μmol/L; Tsat: 12.0-45.0%; ferritin: 15.0-165 μg/L; sTfR: 0.74-2.39 mg/L; zinc: 10.0-20.0 μmol/L; CRP: 0.11-4.52 mg/L

Nutrient reference values – iron EAR: 8.00 mg/day, iron RDI: 18.0 mg/day; zinc EAR: 6.50 mg/day, zinc RDI: 8.00 mg/day

†Natural log transformation performed on the ferritin, sTfR-F and CRP variables

responders: 27.0 (133) vs responders: 69.0 (28.0) μg/L; p=0.053] and significantly lower sTfR [non-responders: 1.73±0.72 vs responders: 1.59±0.93 mg/L; p=0.011]. By 12 months, the tendency for those with ≥10% weight loss to show a more favourable iron profile was only apparent in LP responders who had higher serum iron (non-responders: 13.5±6.3 vs responders: 20.0±12.7 μmol/L; p=0.002), transferrin saturation (non-responders: 19.4± 9.1%; responders: 29.8±20.3%; p=0.001) and lower sTfR (non-responders: 1.92±0.68 vs responders: 1.24±0.52 mg/L; p=0.034). Plasma zinc differed minimally between response groups in both diets. The HP diet appeared to have a greater impact on CRP reduction with successful weight loss than LP (p=0.085 at 12 months), although not significantly so. Similar to the between-diet analysis, contraceptive medication was a significant confounder for transferrin saturation (p=0.031) in the HP diet and for serum iron (p=0.001), transferrin saturation (p=0.001) in the LP diet group.

DISCUSSION

This study evaluated the impact of two energy-restricted diets with contrasting protein, haem iron and zinc content on their associated micronutrient and inflammatory markers in young women. Results showed that during medium to longer term energy restriction, the HP diet had a propensity for increasing iron stores more readily although ferritin and zinc was maintained within normal limits for most individuals on both diets. Responders on the LP diet who lost ≥10% of initial weight also showed a more favourable iron profile compared to non-responders who lost <5% of initial weight.

Iron and zinc homeostasis in adults is essential for tissue oxygen delivery, cell growth and immune function.[43-45] Inadequate body iron impacts adversely on physical performance, work productivity and cognitive function,[43, 44] while zinc deficiency impairs immunity, memory and causes taste disturbances.[45] The tendency for greater iron stores (ferritin) in the HP diet group was comparable to a similar study conducted in middle-aged women.[29] The substantial proportion of individuals identified with iron deficiency (17%) at recruitment also highlights the clinical relevance of HP diets for maintaining iron status, particularly in young women with higher requirements for iron.

Meeting age and gender-appropriate nutrient recommendations can be difficult for young women undergoing energy restriction.[3] This was reflected in our study whereby the HP and LP diets did not reach RDIs for iron and/or zinc, even after detailed modelling and manipulation of the meal plans. In women with greater nutritional needs such as those who experience high menstrual iron losses,[46] adopting energy-restricted diets may become problematic. The persistence and new development of iron and zinc deficiency in a number of our participants was similar to a previous study describing an increase in nutrient deficiency with weight reduction via a nutritionally-complete formula diet.[47] Together with the decline in plasma zinc observed throughout the trial, these outcomes highlight the importance of careful nutritional planning and monitoring during dietary weight management in young women, particularly if energy restriction extends beyond 12 months.

Contrary to our expectations, mean CRP and hepcidin concentration were not greatly elevated at baseline, which was most likely due to the modest obesity and absence of comorbidities in this cohort.[48] Hence, diet therapy and weight loss would only have brought about a minor (if any) decrease in these markers. This finding indicates that unlike previous studies,[18,49] significant weight loss associated reduction of hepcidin may only occur in individuals who are severely obese and/or burdened with

Table 3. Comparison of biochemistry and micronutrient intake between non-responders (<5% loss of initial weight) and responders (≥10% loss of initial weight) within the diets at six and 12 months

Biochemical or intake variable	HP diet						LP diet					
	Weight loss at 6 months		*p* value	Weight loss at 12 months		*p* value	Weight loss at 6 months		*p* value	Weight loss at 12 months		*p* value
	<5% (*n*=8)	≥10% (*n*=10)		<5% (*n*=6)	≥10% (*n*=9)		<5% (*n*=10)	≥10% (*n*=3)		<5% (*n*=8)	≥10% (*n*=4)	
Hb (g/L)	128 ± 11	129 ± 9	0.590	131 ± 14	133 ± 10	0.840	129 ± 7	134 ± 7	0.613	127 ± 11	129 ± 6	0.060
Serum iron (µmol/L)	13.6 ± 8.5	15.8 ± 5.0	0.400	16.7 ± 6.3	14.1 ± 3.3	0.769	14.5 ± 3.7	17.0 ± 4.6	0.453	13.5 ± 6.3	20.0 ± 12.7	0.002
Tsat (%)	19.6 ± 11.8	25.5 ± 9.1	0.878	24.7 ± 10.5	23.2 ± 5.7	0.608	21.9 ± 7.0	25.3 ± 9.0	0.161	19.4 ± 9.1	29.8 ± 20.3	0.001
Serum ferritin (µg/L)†	42.0 (77.0)	59.5 (91.0)	0.137	51.0 (75.0)	70.0 (104.0)	0.172	27.0 (133.0)	69.0 (28.0)	0.053	27.0 (52.0)	51.5 (16.0)	0.263
<15.0 µg/L (%)	0.00	0.00		33.3	0.00		0.00	0.00		50.0	0.00	
sTfR (mg/L)	1.47 ± 0.37	1.32 ± 0.25	0.241	1.48 ± 0.31	1.32 ± 0.26	0.658	1.73 ± 0.72	1.59 ± 0.93	0.011	1.92 ± 0.68	1.24 ± 0.52	0.034
sTfR-F index†	1.03 (0.92)	0.76 (0.40)	0.019	1.04 (0.99)	0.63 (0.46)	0.145	1.06 (2.14)	0.62 (0.89)	0.050	1.12 (2.52)	0.67 (0.69)	0.223
Zinc (µmol/L)	13.4 ± 1.5	14.0 ± 2.0	0.585	13.0 ± 2.7	12.4 ± 1.5	0.454	13.3 ± 1.6	13.3 ± 1.1	0.842	12.0 ± 1.3	11.9 ± 1.7	0.660
<10.0 µmol/L (%)	0.00	0.00		16.7	0.00		0.00	0.00		0.00	25.0	
CRP (mg/L)†	5.43 (14.69)	0.92 (12.67)	0.145	3.78 (17.33)	1.06 (25.57)	0.085	2.17 (13.82)	5.16 (21.16)	0.310	3.45 (11.97)	5.14 (7.74)	0.830
>10.0 mg/L (%)	0.25	10.0		16.7	11.1		20.0	33.3		12.5	0.00	
Iron intake (mg/day)	11.8 ± 2.5	12.3 ± 3.4	0.953	12.3 ± 2.9	11.0 ± 1.1	0.244	8.9 ± 1.8	7.7 ± 1.9	0.299	8.7 ± 0.9	7.9 ± 1.8	0.911
<EAR (%)	0.00	0.00		0.00	0.00		10.0	33.3		25.0	25.0	
<RDI (%)	100	90.0		83.3	88.9		90.0	100		87.5	100	
Zinc intake (mg/day)	11.9 ± 1.5	13.4 ± 3.0	0.586	12.6 ± 1.1	11.8 ± 1.5	0.254	6.7 ± 1.3	7.7 ± 1.5	0.980	6.9 ± 0.7	7.6 ± 1.2	0.783
<EAR (%)	0.00	0.00		0.00	0.00		40.0	0.00		25.0	25.0	
<RDI (%)	0.00	0.00		0.00	0.00		80.0	66.7		75.0	75.0	

Mean ± SD or median (range). HP, higher-protein; LP, lower-protein; Tsat, transferrin saturation; sTfR, soluble transferrin receptor; sTfR-F, soluble transferrin receptor-ferritin index; CRP, C-reactive protein; EAR estimated average requirement; RDI, recommended dietary intake

Reference ranges – Hb: 115-165 g/L; serum iron: 10.0-30.0 µmol/L; Tsat: 12.0-45.0%; ferritin: 15.0-165 µg/L; sTfR: 0.74-2.39 mg/L; zinc: 10.0-20.0 µmol/L; CRP: 0.11-4.52 mg/L

Nutrient reference values – iron EAR: 8.00 mg/day, iron RDI: 18.0 mg/day; zinc EAR: 6.50 mg/day, zinc RDI: 8.00 mg/day

†Natural log transformation performed on the ferritin, sTfR-F and CRP variables

comorbid conditions and greater levels of inflammation.[12,18,50] Despite low baseline hepcidin, higher circulating iron levels were still observed in LP responders who lost ≥10% of weight. While a superior iron profile in LP responders vs. non-responders was likely to be the result of better diet quality and compliance, these findings do not rule out a possible standalone benefit of weight loss on iron status that is independent of significant hepcidin reduction. Higher haem iron intake may have masked the iron-related benefits of weight loss, which explains the absence of significant differences between response groups in the HP diet.

This study enrolled young women, a nutritionally vulnerable population for which limited clinical research is available. Limitations of this study include the high rate of attrition (leading to small sample size) and absence of longitudinal hepcidin assessment. Interestingly, similar difficulties with attrition was recently reported in a systematic review of weight management interventions in young adults,[51] highlighting the inherent challenges of conducting research in this population.

In conclusion, this study supports the use of well-designed energy-restricted diets for weight loss and reasonable maintenance of iron and zinc status in young, healthy, overweight and obese women. Despite insignificant changes to inflammatory status, loss of ≥10% initial weight was associated with a superior iron profile which was evident under conditions of limited haem iron intake. This may reflect greater diet quality and compliance, or the possible influence of iron regulatory pathway(s) independent of significant inflammatory hepcidin reduction.

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AUTHOR DISCLOSURES

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Original Article

Impact of diet and weight loss on iron and zinc status in overweight and obese young women

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飲食和減重對過重和肥胖年輕女性鐵和鋅狀況之影響

鐵和鋅缺乏常見於年輕過重女性。本研究旨在評估，年輕婦女參與 12 個月減重試驗期間之鐵、鋅和發炎狀況。參與婦女(18-25 歲; BMI ≥27.5 kg/m^2)隨機分為較高蛋白(HP: 含 32%蛋白質; 鐵 12.2 mg/日; 鋅 11.7 mg/日)或較低蛋白飲食(LP: 含 20%蛋白質; 鐵 9.9 mg/日; 鋅 7.6 mg/日)兩組，兩種飲食所含之血基質鐵和鋅含量不同。在完成試驗者中，雖然兩組皆落在正常範圍內，但 HP 組有較高的儲鐵蛋白中位數(52.0 比 39.0 μg/L; p =0.021)及較低的可溶性轉鐵蛋白受體-儲鐵蛋白指數中位數(sTfR-F; 0.89 比 1.05; p =0.024)。在基線時，C 反應蛋白(CRP; HP: 3.54; LP: 4.63 mg/L)和鐵調節素(HP: 5.70; LP: 8.25 ng/mL)中位數值未升高，且兩組間的鋅與 C 反應蛋白濃度並未隨著試驗進行而有顯著差異。HP 組體重減少≥10%者，比起減少<5%者，在第 6 個月時有較低的 sTfR-F 中位數(0.76 比 1.03; p=0.019)。在第 12 個月時，LP 組中體重減少≥10%者，比起減少<5%者，其鐵狀況受到影響較明顯，例如有較高平均血清鐵(20.0 比 13.5 μmol/L; p=0.002)、轉鐵蛋白飽和度(29.8%比 19.4%; p=0.001)和較低的 sTfR (1.24 比 1.92 mg/L; p=0.034)。結果顯示，為期 12 個月之熱量限制仍舊可以維持正常鐵和鋅濃度。在基線時未發炎和鐵調節素未升高的情況下，體重減少≥10%者呈現較佳鐵指標數值，也許可反映出，這些參與者具較高順從性，或是鐵調節機制潛在影響與發炎性鐵調節素的減少無關。

關鍵字：肥胖、體重減少、年輕成人、鐵、鋅