Original Research

Can Dietary Treatment of Non-Anemic Iron Deficiency Improve Iron Status?

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Objective: To investigate the efficacy of, first, a dietary regimen involving increased consumption of iron-rich foods and enhancers of iron absorption and decreased consumption of inhibitors of iron absorption and, second, a low dose iron chelate iron supplement, for increasing iron stores in young adult New Zealand women with mild iron deficiency (MID).

Methods: The study was a 16 week randomized placebo-controlled intervention. Seventy-five women aged 18 to 40 years with MID (serum ferritin <20 μg/L and hemoglobin ≥ 120 g/L) were assigned to one of three groups: Placebo, Supplement (50 mg iron/day as amino acid chelate) or Diet. Participants in the Diet Group were given individual dietary counseling to increase the intake and bioavailability of dietary iron. Dietary changes were monitored by a previously validated computer-administered iron food frequency questionnaire.

Results: Diet Group members significantly increased their intake of flesh foods, heme iron, vitamin C and foods cooked using cast-iron cookware and significantly decreased their phytate and calcium intakes. Serum ferritin increased in the Supplement and Diet Groups by 59% ($p=0.001$) and 26% ($p=0.068$), respectively, in comparison to the Placebo Group. The serum transferrin receptor:serum ferritin ratio decreased by 51% in the Supplement Group ($p=0.0001$), and there was a non-significant decrease of 22% ($p=0.1232$) in the Diet Group.

Conclusions: This study is the first, to our knowledge, to demonstrate that an intensive dietary program has the potential to improve the iron status of women with iron deficiency.

INTRODUCTION

Iron supplementation is accepted as the most appropriate way to improve iron status in iron deficiency anemia [1]. The treatment of non-anemic iron deficiency states, however, is much more controversial. Women are commonly advised to increase their dietary intake of readily available iron if they have concerns about their iron status [2]. This advice is based on studies showing substantial increases in iron absorption when flesh foods and foods containing ascorbic acid are consumed and substantial decreases in iron absorption when foods containing inhibitors of iron absorption such as phytate, calcium, tea and coffee are consumed with meals [3]. An increase in the intake of fortified foods [4] and foods cooked in cast iron cookware [5] would also be expected to result in a higher intake of available iron. While numerous studies have demonstrated effects of increased levels of these food components on iron absorption from single meals, recent studies have suggested that at least some of these effects may be far smaller in the whole diet [6, 7]. To our knowledge there are no reports that describe the use of diet to improve the iron status of women who are iron deficient, although some have assessed the effects

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Abbreviations: BLU=Blood Loss Units, Iron FFQ=Iron Food Frequency Questionnaire, MID=Mild Iron Deficiency, sTfR:SF ratio=Serum transferrin receptor: serum ferritin ratio.

of dietary change on iron status in women who are largely iron-sufficient [8, 9].

There is a need to test the efficacy of dietary strategies for treating non-anemic iron deficiency because this condition is commonplace in industrialized nations. For example, 6% of women of child-bearing age in the NHANES III study [10] and 4% in the New Zealand National Nutrition Survey [11] had non-anemic iron deficiency defined as a serum ferritin concentration of less than 12 μg/L in the absence of anemia. The percentage of non-anemic women with a serum ferritin concentration of less than 20 μg/L, the level at which adverse functional effects have been reported [12] is even higher.

The aim of the study, therefore, was to investigate the efficacy of, first, a dietary regimen involving increased consumption of iron-rich foods and enhancers of iron absorption and decreased consumption of inhibitors of iron absorption and, second, a low dose iron chelate iron supplement, for increasing iron stores in young adult New Zealand women with mild iron deficiency (MID).

METHODS

Study Population

Seventy-five women (68 omnivorous, 7 lacto-ovo-vegetarian) with mild iron deficiency defined as serum ferritin <20 μg/L and hemoglobin ≥ 120 g/L, ages 18 to 40 years, were recruited from the greater Dunedin (New Zealand) area. Approximately half were recruited from a cross-sectional study designed to examine the etiological factors associated with MID [13]. The remainder of the participants were recruited by advertisement in local newspapers. Exclusion criteria were pregnancy or lactation, irregular menstruation, health problems likely to influence iron status (for instance gastrointestinal disease), medication likely to affect iron status [14], anorexia nervosa or bulimia [15] and veganism. Participants were asked not to take iron, vitamin C or calcium supplements during the study or to donate blood.

Ethical approval for this study was granted by the Southern Regional Health Authority Ethics Committee Otago. All participants provided informed written consent.

Study Design

The study was a 16-week, double-blind, placebo-controlled intervention trial. At baseline, participants were assigned to one of three groups using a randomized stratified block design: placebo and usual diet (Placebo Group), iron supplement and usual diet (Supplement Group) or dietary change and placebo (Diet Group). The following strata were used for balancing the groups: recruitment serum ferritin (<12 μg/L or 12–19 μg/L), menstrual blood loss (<33.5 blood loss units (BLU) or ≥ 33.5 BLU (based on the median value for a group of young New Zealand women [16])) and vegetarianism (yes or no). Participants attended monthly clinics at which they provided a fasting morning venipuncture blood sample (17 mL) for determination of hemoglobin, serum ferritin, serum transferrin receptor and C-reactive protein. Participants were asked questions at each monthly appointment to monitor their adherence to the exclusion criteria.

Tablets

Participants were asked to take one tablet each day with a meal. The iron supplement was an amino acid chelate (bisglycino iron II) providing 50 mg of elemental iron (FerroChel (Albion Laboratories, Inc., Clearfield, Utah)) [17]. The placebo tablets were malto-dextrin. Analysis in our laboratory (AOAC method 944.02) indicated that each iron tablet contained 47 mg of iron and each placebo tablet 0.7 μg of iron. Iron and placebo tablets were prepared and packaged by the same company (Albion Laboratories, Inc., Clearfield, Utah) and were identical in appearance (dark green opaque gel capsules) and dose regimen. Only the research dietitian, who instructed and supported the participants during the study, was aware of the treatment allocation.

Dietary Advice

Participants in the Diet Group were given individual dietary advice by a New Zealand Registered Dietitian to increase their iron intake and to increase the bioavailability of that iron. Advice was based on quantitative information from their Iron Food Frequency Questionnaires (Iron FFQs) and additional information obtained during the interview about their eating habits and other factors that would influence their ability to make the necessary changes to their eating pattern. Participants in the Diet Group were provided with recipe books, sufficient fruit juice to increase their intake by one serving a day (250 mL of juice containing 30 mg/dL vitamin C to be consumed with meals) and a cast-iron frypan to use when cooking tomato-based sauces. Participants attended diet counseling sessions with the Dietitian at baseline and at weeks 2 and 6. Telephone check-ups were carried out at weeks 10 and 14.

The dietary intervention was based on the following principles:

1. *Increasing the intake of iron containing foods.* Participants were encouraged to eat at least one serving of high iron foods rich in heme iron (e.g., red meat, liver) and one serving of medium iron food (e.g., processed meat, chicken, fish, legumes) each day. Vegetarians were advised to eat at least four servings of medium iron rich foods each day. In addition, participants were encouraged to increase their intake of foods with a high non-heme iron, fortificant iron or vitamin C content and to use a cast-iron fry pan, especially when cooking tomato-based sauces.
2. *Increasing the intake of foods containing factors said to*

enhance non-heme iron absorption. Participants were asked to consume foods and beverages containing at least 50 mg of vitamin C in each meal. Tables showing quantities of foods providing 50 mg of vitamin C, recipes using vitamin C-rich foods and practical ideas for conserving the vitamin C content of fruit and vegetables were provided. Participants were also given ideas for adding meat, fish and chicken to composite dishes based on plant foods. The use of fermented soy sauce was also encouraged.

3. *Decreasing the intake of foods containing factors believed to inhibit non-heme iron absorption.* Participants were given advice to decrease their intake of foods that are particularly high in phytates (e.g., wheat bran, nuts) and polyphenols (e.g., spinach). They were also asked to soak dried beans and discard the soaking water before cooking and to choose beans other than soybeans.
4. *Modifying eating patterns so that enhancers of non-heme iron absorption were eaten with meals and potential inhibitors between meals.* Participants were advised to consume tea, coffee, red wine and port between meals, if at all, replacing them with vitamin C-rich drinks with meals. While participants were also asked to avoid large servings of calcium-rich foods with their main meals, care was taken to encourage adequate calcium intakes between meals to ensure that this advice did not compromise their calcium intake.

The dietary advice was designed to improve iron status within the context of a healthy diet that was in keeping with the New Zealand Food and Nutrition Guidelines [2].

Adherence

Adherence to the tablet regimen was enhanced by discussing strategies with the participant to minimize any barriers to taking the tablets, choosing a tablet with few side effects and warning participants about possible side effects [18]. In addition, tablet packets were marked with the days of the week [19], and participants recorded their consumption on special calendars [20].

Apart from the drinks and frying pan which participants were provided with, all foods were purchased and prepared by the participants themselves. Adherence to the diet regimen was enhanced by a Registered Dietitian providing counseling and ongoing support for the dietary changes, giving individualized advice and discussing strategies with the participant to minimize barriers to following the diet principles. In addition, participants were provided with a 250 mL pack of fruit juice per day (Frucor, Auckland, New Zealand) and a vitamin-C rich fruit syrup (Barker Fruit Processors Ltd, Geraldine, New Zealand)), recipe books (Pork Board, New Zealand; Beef & Lamb Marketing Bureau, New Zealand) and a cast-iron frying pan.

Adherence to the intervention was assessed by taking a tablet count at each meeting with the dietitian, checking the calendar report of the number of tablets taken and administering the computerized Iron Food Frequency Questionnaire at recruitment and weeks 4, 8 and 16.

Laboratory Procedures

The venipuncture blood samples were refrigerated immediately following collection and the serum separated and frozen within four hours at −20°C. All serum samples collected for the same individual over the 16-week period were analyzed in the same batch to eliminate inter-assay variability. Hemoglobin was measured by the cyanmethemoglobin method [21]. Serum ferritin and serum transferrin receptor were assessed by ELISA procedures using commercial kits (Ramco Laboratories Inc, Houston, Texas). C-reactive protein in serum was measured by kinetic turbidimetry using the Behring Turbitimer (Behringwerke AG, Marburg, Germany).

Serial replications of quality control sera for serum ferritin (Ramco Laboratories Inc, Houston, Texas) and serum transferrin receptor (Ramco Laboratories Inc, Houston, Texas) were used to check the precision and accuracy of the analytical methods. The mean (SD) values for the quality control sera for serum ferritin were 12.4 (1.0) μg/L (CV: 7.7%, n=18) and 67.5 (5.3) μg/L (CV: 7.8%, n=18) compared to the manufacturer's specified acceptable ranges of 12.6 (7.6 to 17.6) μg/L and 64.4 (52 to 76.8) μg/L. Corresponding quality control sera values for the serum transferrin receptor were 5.3 (1.0) mg/L (CV: 18.3%, n=15) and 11.1 (1.2) mg/L (CV: 10.4%, n=15) compared with the manufacturer's acceptable ranges of 4.4 to 7.5 mg/L and 9.6 to 15.7 mg/L.

Assessment of Dietary Intake

A previously validated computer-administered Iron Food Frequency Questionnaire (Iron FFQ) was used to estimate usual dietary intake over one month of non-heme, heme and total iron and selected modifiers of iron absorption: meat/fish/poultry, phytate, calcium, vitamin C, tea and coffee [22]. The Iron FFQ was administered at recruitment and at weeks 4, 8 and 16. Heme iron was estimated according to Rangan *et al.* [23] as 50% to 70% of the total iron in beef, lamb, pork and chicken and approximately 35% of the total iron in sausages and liver.

Collection of Demographic and Health Status Data

Demographic data, as well as data on blood loss due to menstruation, blood donation and nose bleeds, were collected via a pre-tested questionnaire at screening. Menstrual blood loss was determined via a validated menstrual blood loss questionnaire described earlier [16] and measured in Blood Loss Units (BLU).

Statistical Analysis

SPSS for Macintosh Version 6.1.1 was used to carry out all statistical analyses. The baseline characteristics of the participants were compared using one-way ANOVA with Dunnett's

procedure as a *post hoc* comparison for continuous variables [24] or the chi-square test for dichotomous variables. Otherwise, statistical comparisons were made between each intervention group and the Placebo Group as advocated by Senn and Auclair [25], rather than within groups over time.

Because individuals with infection are likely to have falsely elevated serum ferritin concentrations [26], the baseline serum ferritin concentration of participants with a serum C-reactive protein concentration greater than 10 mg/L [27] (n=3) was replaced with their serum ferritin value at week 4. None of the participants had an elevated C-reactive protein at the final blood test.

Multiple linear regression was used to examine the difference in dietary and biochemical variables between each intervention group and the Placebo Group during the study, adjusted for age and the baseline value. Age was controlled for because, although there was no statistically significant difference in the ages of the three groups at baseline, there was a five-year difference between those in the Placebo Group and those in the Diet Group. For the biochemical indices the outcome variable was the value of the index at week 16. For the dietary variables, the outcome variable was mean dietary intake during the study based on the results of the Iron FFQs at weeks 4, 8 and 16. Logistic regression was used to determine whether there was any change in the dichotomous variable "cast-iron cookware use" in the intervention groups compared to the Placebo Group.

Arithmetic means and the difference between the mean for the intervention group and the mean for the Placebo Group are quoted for normally distributed variables. Geometric means and the ratio of the geometric mean for the intervention group and the geometric mean for the Placebo Group are quoted for the variables which were log transformed.

Confidence intervals were calculated for the difference, or ratio, between the intervention group and the Placebo Group outcomes to assess the clinical importance of the range of plausible values [28]. A confidence interval for an adjusted difference that includes the value 0 indicates that there is no significant difference between the intervention group and the Placebo Group outcome. A confidence interval for an adjusted ratio that includes the value 1 indicates that there is no significant difference between the intervention group and the Placebo Group outcome.

RESULTS

Eight participants withdrew from the study (4 from the Placebo Group, 6 from the Supplement Group, 1 from the Diet Group). Ten participants were excluded during the study. Eight individuals were excluded because they became anemic: five became anemic between recruitment and the start of the study, three became anemic during the study (one from the Placebo Group, two from the Diet Group). In addition, one participant was excluded because she made a blood donation and one because she became pregnant. There was no significant difference between groups in the number of withdrawals and exclusions. The Placebo Group, therefore, comprised 19 individuals, the Supplement Group 16 and the Diet Group 22.

At baseline there was no significant difference between the Placebo Group and the two treatment groups for hemoglobin, serum ferritin or serum transferrin receptor, blood loss variables such as menstrual blood loss, Quetelet's body mass index or the dietary variables of interest (Tables 1 and 2).

There was 97% compliance with tablet taking in the Supplement Group. There was no change in the dietary intake of individuals in the Supplement Group compared to the Placebo Group (Table 3). Members of the Diet Group significantly increased their intake of meat/fish/poultry, heme iron and vitamin C and their use of cast-iron cookware and significantly decreased their intake of phytate and calcium and their phytate: iron molar ratio (Table 3). There was no significant change in total iron intake. The Diet Group reported a 31 g/day higher

Table 1. Non-Dietary Characteristics of the Participants at Baseline

	Placebo Group (n=19)	Supplement Group (n=16)	Diet Group (n=22)
Age (years)[1]	25.4 (6.2)	27.2 (8.0)	30.8 (7.8)
Hemoglobin (g/L)[1]	131.6 (6.7)	130.9 (8.8)	131.6 (7.2)
Serum ferritin (μg/L)	11.7 (9.9 to 13.7)	8.4 (6.7 to 10.7)	10.3 (8.3 to 12.7)
Transferrin receptor (mg/L)	4.9 (4.4 to 5.4)	5.2 (4.1 to 6.6)	5.8 (5.2 to 6.5)
Menstrual blood loss (BLU)	44.2 (32.1 to 60.9)	48.9 (32.7 to 73.1)	42.9 (29.9 to 61.8)
Has children (%)	11	25	50
Blood donor (%)	37	19	32
Has nose bleeds (%)	32	38	27
Body mass index[2]	24.5 (22.1 to 27.1)	22.5 (20.5 to 24.7)	23.8 (22.4 to 25.2)

Values are geometric means (confidence intervals) unless stated otherwise.

[1] Arithmetic mean (SD).

[2] Quetelet's body mass index (m/kg^2).

BLU=Blood loss units.

There were no statistically significant differences between the intervention groups and the Placebo Group.

Table 2. Dietary Characteristics of the Participants at Baseline

	Placebo Group (n=19)	Supplement Group (n=16)	Diet Group (n=22)
Meat/fish/poultry intake (g)[1]	83.5 (51.4)	133.8 (57.5)	117.7 (75.0)
Total iron (mg)	9.9 (8.6 to 11.5)	11.1 (9.8 to 12.5)	10.5 (9.1 to 12.0)
Heme iron (mg)	0.8 (0.5 to 1.1)	1.8 (1.3 to 2.4)	1.4 (1.0 to 1.8)
Non-heme iron (mg)	9.3 (8.1 to 10.8)	9.7 (8.6 to 11.1)	9.4 (8.2 to 10.8)
Vitamin C (mg)	87 (56 to 133)	98 (78 to 125)	88 (70 to 109)
Phytate (mg)	992 (739 to 1330)	1090 (820 to 1450)	1048 (760 to 1446)
Phytate: iron molar ratio	8.5 (6.5 to 11.0)	8.3 (6.5 to 10.6)	8.5 (6.6 to 11.0)
Calcium (mg)	735 (611 to 885)	582 (501 to 677)	697 (592 to 820)
Uses cast-iron cookware (%)	26.3	25.0	9.1

Values are geometric means (confidence intervals) unless stated otherwise.

[1] Arithmetic mean (SD).

There were no statistically significant differences between the intervention groups and the Placebo Group.

meat/fish/poultry intake during the study than the Placebo Group (controlling for differences in baseline intake). This is equivalent to approximately one chicken drumstick, half a sausage or quarter of a cup of lean beef mince [29] or an additional one third of a serving of meat each day [2]. The Diet Group reported a 136 mg higher vitamin C intake during the study than the Placebo Group (controlling for differences in baseline intake). This is equivalent to approximately two cups of fruit juice, one and a half kiwifruit or two oranges [29] and is more than the researchers supplied to the participants.

Both intervention groups showed an improvement in serum ferritin after 16 weeks, when compared to the Placebo Group (Table 4). The Diet Group experienced a 26% increase ($p=0.068$) and the Supplement Group a 59% increase ($p=0.001$) in serum ferritin. There was no change in hemoglobin in either intervention group. Serum transferrin receptor concentration decreased in the Supplement Group (17%), but was unchanged in the Diet Group. The serum transferrin receptor:serum ferritin ratio decreased 51% in the Supplement Group ($p=0.0001$), with a non-significant 22% decrease in the Diet Group.

DISCUSSION

The Women's Iron Study is the first study to demonstrate both that women are able to make significant changes to their dietary intake of flesh foods, heme iron, vitamin C, phytate, calcium, and foods cooked using cast-iron cookware in a free-living situation and that dietary change can improve the iron status of women with iron deficiency.

It is not possible to identify which specific components of the dietary intervention in this present study were responsible for the (marginally significant) improvement in iron status in the Diet Group. However, an increase in both the heme iron content of the diet and the bioavailability of its non-heme iron almost certainly played a role. Strategies focusing on the bioavailability of non-heme iron are of particular interest in this

Table 3. Mean Dietary Intake of Total, Heme and Non-Heme Iron, and Iron Absorption Modifiers in the Placebo and Intervention Groups during the Study (Weeks 4, 8 and 16)

	Placebo (n=19)	Supplement[2] (n=16)	Diet (n=22)	Adjusted Ratio (95% CI)[1]	
				Supplement *vs.* Placebo	Diet *vs.* Placebo
Meat/fish/poultry (g)[3]	89.0	128.5	141.5	0.96 (0.74 to 1.24)	1.28 (1.01 to 1.63)
Total iron (mg)	11.0	11.1	12.4	0.96 (0.82 to 1.17)	1.10 (0.94 to 1.27)
Heme iron (mg)	0.9	1.4	1.9	0.91 (0.77 to 1.07)	1.23 (1.06 to 1.43)
Non-heme iron (mg)	10.3	9.9	11.0	0.94 (0.80 to 1.11)	1.05 (0.90 to 1.23)
Vitamin C (mg)	98.7	95.8	235.0	0.91 (0.69 to 1.19)	2.37 (1.83 to 3.07)
Phytate (mg)	1109.2	1262.1	818.7	1.06 (0.80 to 1.42)	0.72 (0.54 to 0.95)
Phytate: iron molar ratio	8.5	9.7	5.6	1.15 (0.88 to 1.51)	0.67 (0.52 to 0.87)
Calcium (mg)	793.8	654.6	624.9	0.90 (0.76 to 1.07)	0.79 (0.67 to 0.92)
Uses cast-iron cookware (%)	26	25	86*		

Values are geometric means unless stated otherwise.

[1] Ratio of the geometric mean for the intervention group to the geometric mean for the Placebo Group during the study adjusted for age and baseline value.

[2] Average iron from the supplement was 45.6 mg/day.

[3] Arithmetic means.

[*] Statistically significant difference ($p=0.04$) between the intervention group and the Placebo Group.

Table 4. Mean Hemoglobin, Serum Ferritin and Serum Transferrin Receptor Concentrations during the Study

	Week					Adjusted Ratio (95% CI)[1]
	0	4	8	12	16	
Placebo Group (n=19)						
Hemoglobin (g/L)[2]	131.6	133.6	133.6	132.6	133.0	
Serum ferritin (μg/L)	11.7	13.4	12.2	12.4	12.6	
Transferrin receptor (mg/L)	4.9	4.9	5.0	5.0	5.1	
sTfR:SF[3] ratio	419.4	366.3	449.2	415.3	401.2	
Supplement Group (n=16)						
Hemoglobin (g/L)[2]	130.9	132.8	133.1	135.8	133.4	1.01 (0.98 to 1.03)
Serum ferritin (μg/L)	8.4	12.1	13.3	14.7	16.8	1.59 (1.22 to 2.07)
Transferrin receptor (mg/L)	5.2	5.2	4.6	4.5	4.3	0.83 (0.71 to 0.97)
sTfR:SF ratio	609.8	401.7	302.2	312.1	258.6	0.49 (0.35 to 0.69)
Diet Group (n=22)						
Hemoglobin (g/L)[2]	131.6	132.7	132.3	132.9	132.2	0.99 (0.97 to 1.01)
Serum ferritin (μg/L)	10.3	10.7	11.7	11.9	14.0	1.26 (0.98 to 1.60)
Transferrin receptor (mg/L)	5.8	5.6	5.3	5.6	5.5	0.96 (0.82 to 1.12)
sTfR:SF ratio	564.0	524.1	451.5	472.2	393.5	0.78 (0.56 to 1.07)

Values are geometric means unless stated otherwise.

[1] Ratio of the geometric mean for the intervention group to the geometric mean for the Placebo Group at week 16 adjusted for age and baseline value.

[2] Arithmetic means.

[3] Serum transferrin receptor: serum ferritin ratio.

study given the small increase in the intake of flesh foods (31 g per day). We have estimated that 47% of the heme iron consumed by the Diet Group was absorbed, based on their baseline mean serum ferritin value of 10.3 μg/L and a regression line for heme iron absorption derived by Hallberg *et al.* [30]. The Diet Group consumed 1.9 mg of heme iron per day on average during the intervention, an increase of 0.36 mg over the baseline value. This is equivalent to an additional 0.17 mg of absorbed iron per day, or 19.0 mg of iron during the course of the 16 week study. Such an increase is still substantially lower than the additional 29 mg of storage iron (calculated on an assumption that 1 μg of serum ferritin may correspond to 10 mg of storage iron in healthy adults [31]) implied by our observed rise in serum ferritin of 2.9 μg/L. Based on these calculations, some characteristic of the dietary intervention, in addition to its heme iron content, must have contributed to the increase in serum ferritin concentrations.

Single meal studies have shown that adding flesh foods to a basal meal that does not contain absorption enhancers substantially improves non-heme iron absorption [32], but adding flesh foods to a meal which is already high in vitamin C may be less effective. Layrisse *et al.* [33] found that adding 66 mg of ascorbic acid (as papaya) to 100 g of cooked maize resulted in an absorption rate for non-heme iron of 26.8%, but there was no additional increment when 100 g of meat/fish/poultry (as fish) was added to the maize and papaya meal (non-heme iron absorption 24.9%). Because the increase in meat/fish/poultry intake in our study occurred against a background of markedly increased vitamin C intakes, it is not possible to decide whether the increase in either one or both of these enhancers of non-heme absorption contributed to the improved iron status of those in the Diet Group. A recent study by Cook and Reddy [6] confirms the enhancing effect of both vitamin C and animal tissue on iron absorption from the complete diet, but only 8% and 4% of the total variation in iron absorption was explained by these food components respectively. Interestingly, in the same study, phosphorus (a component of phytate but widely distributed in foods) accounted for 19% of the variation in iron absorption. Although members of the Diet Group also decreased their intake of phytate, and their phytate:iron molar ratio, during the study, their intake of phytate remained high.

The Women's Iron Study has demonstrated that a 16-week dietary intervention can improve iron status in women with mild iron deficiency. However, dietary change may not be the most effective treatment for mild iron deficiency states in the general public. The women taking part in this study received an intensive and expert individualized dietary program, yet they achieved only a small increase in their intake of flesh foods (31 g per day). Moreover, two individuals in the Diet Group became anemic during the study. Therefore, while the highly motivated person may be able to use diet to improve iron status, supplementation is likely to be a more practical option for most premenopausal women with mild iron deficiency.

The amino acid chelate used in this study has been shown earlier to be effective for treating iron deficiency anemia [17]. Although ferrous sulphate (100 mg elemental iron) is the iron supplement most commonly prescribed to New Zealand women, the chelate was selected for this study because it is better tolerated [17, 34] and therefore more likely to have a higher compliance rate. All participants kept a daily log of symptoms, including abdominal discomfort, bloating, constipation, diarrhea, nausea, vomiting, headache and fatigue [34]. When symptoms occurring immediately before or during menses were excluded, there were no significant differences in

either the frequency or severity of the symptoms experienced by those taking the supplement, compared to those taking the placebo tablets. Moreover, the amino acid chelate is available in a lower dose so was less likely to impair zinc absorption [35]. The results presented here demonstrate that the chelate is also effective for treating non-anemic iron deficiency states. Average serum ferritin concentration doubled to 17 μg/L in the iron supplemented group, and the serum transferrin receptor: serum ferritin ratio dropped below 500 (the proposed cutoff indicating fully depleted iron stores [36]) by the end of the study. Moreover, neither of these parameters had reached a plateau during the four month intervention period, suggesting that they would probably continue to improve over a longer supplementation period.

Widely accepted reference ranges for serum transferrin receptor concentration for women of child-bearing age have not yet been determined in New Zealand or elsewhere. Therefore, a "normal" reference range for serum transferrin receptor was developed using serum transferrin receptor values for 31 healthy Caucasian women aged 18 to 40, who were neither pregnant nor lactating, had no biochemical evidence of infection (using a stringent C-reactive protein cutoff of <5 mg/L) and were not iron deficient (serum ferritin ≥ 53 μg/L (the 75th percentile for white women 20 to 40 years of age in the NHANES II reference sample [37], hemoglobin ≥ 120 g/L). Their hemoglobin and serum ferritin concentrations were normal (mean (SD): 133.8 (3.1) mg/L and 75.0 (14.7) μg/L, respectively) and their serum transferrin receptor concentration ranged between 3.18 and 6.99 mg/L with a mean (SD) of 5.10 (1.0) mg/L, yielding a serum transferrin receptor:serum ferritin ratio of 70.7. This mean serum transferrin receptor concentration is slightly lower but in general agreement with values for healthy non-anemic North American populations determined using an equivalent ELISA method: 5.36 (0.62) mg/L (38) and 5.56 (1.44) mg/L [39].

Serum transferrin receptor concentration decreased significantly in the iron supplemented group indicating that some tissue iron deficit had existed. However, the baseline serum transferrin receptor concentration amongst these mildly iron deficient women (5.20 mg/L) was no different to the mean value for our similar but iron sufficient reference population (5.10 mg/L). Therefore, in the present study, a single measure of serum transferrin receptor concentration was not able to differentiate mildly iron deficient women from women who were iron sufficient.

Skikne *et al.* [36] have proposed a two stage model of iron depletion in which serum transferrin receptor concentrations only increase once the iron stores have been completely depleted (indicated by a serum ferritin of less than 12 μg/L). One would therefore expect that an improvement in the iron status of a group of iron deficient individuals would be marked first by a decrease in serum transferrin receptor concentration as the functional tissue iron compartment was replenished and only then by an increase in serum ferritin concentration as the iron stores improved. In our study, however, iron repletion on supplementation was accompanied by a simultaneous decrease in serum transferrin receptor concentration and increase in serum ferritin concentration, suggesting that the functional tissue and storage compartments may be replenished concurrently. This finding is supported by Zhu *et al.* [40] who have recently reported a simultaneous response of serum ferritin and serum transferrin receptor to iron supplementation amongst women with a non-anemic iron deficiency state.

To our knowledge, this is the first study to test the efficacy of dietary manipulation for treating iron deficiency without anemia. Our findings suggest that a dietary intervention that increases intakes of both heme iron and enhancers of iron absorption such as flesh foods and vitamin C, while at the same time decreasing the intake of phytic acid, may improve the iron status of premenopausal women with low iron stores. The improvements in iron status achieved in response to dietary change are, however, considerably smaller than those possible with iron supplementation. This is, in part, because of the difficulties associated with embarking on, and sustaining, a dietary regimen that requires increased consumption of flesh foods.

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REFERENCES

1. Hoffbrand AV, Pettit JE: "Essential Haematology," 3rd ed. Oxford: Blackwell Scientific Publications, 1993.
2. Nutrition Taskforce to the Department of Health: "Food for Health." Wellington, NZ: Department of Health, 1991.
3. Hurrell RF: Bioavailability of iron. Eur J Clin Nutr 51:S4–S8, 1997.
4. Roe MA, Fairweather-Tait SJ: High bioavailability of reduced iron added to UK flour. Lancet 353:1938–1939, 1999.
5. Adish AA, Esrey SA, Gyorkos TW, Jean-Baptiste J, Rojhani A: Effect of consumption of food cooked in iron pots on iron status and growth of young children: a randomised trial. Lancet 353:712–716, 1999.
6. Cook JD, Reddy MB: Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr 73:93–98, 2001.
7. Reddy MB, Cook JD: Effect of calcium intake on nonheme-iron

absorption from a complete diet. Am J Clin Nutr 65:1820–1825, 1997.
8. Hunt JR, Gallagher SK, Johnson LK, Lykken GI: High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. Am J Clin Nutr 62:621–632, 1995.
9. Lyle RM, Weaver CM, Sedlock DA, Rajaram S, Martin B, Melby CL: Iron status in exercising women: the effect of oral iron therapy vs increased consumption of muscle foods. Am J Clin Nutr 56: 1049–1055, 1992.
10. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL: Prevalence of iron deficiency in the United States. JAMA 277: 973–976, 1997.
11. Ferguson EL, Morrison IM, Faed JM, Parnell WR, McKenzie J, Wilson NC, Russell DG: Dietary iron intakes and biochemical iron status of 15–49 year old women in New Zealand: Is there a cause for concern? NZ Med J 114:134–137, 2001.
12. Rowland TW, Deisroth MB, Green GM, Kelleher JF: The effect of iron therapy on the exercise capacity of nonanemic iron-deficient adolescent runners. Am J Dis Child 142:165–169, 1988.
13. Heath A-LM, Skeaff CM, Williams S, Gibson RS: The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. Pub Health Nutr (in press), 2000.
14. Thomas JA: Drug-nutrient interactions. Nutr Rev 53:271–282, 1995.
15. American Psychiatric Association: "Diagnostic and Statistical Manual of Mental Disorders," 4th ed. Washington, DC: American Psychiatric Association, 1994.
16. Heath A-LM, Skeaff CM, Gibson RS: Validation of a questionnaire method for estimating extent of menstrual blood loss in young adult women. J Trace Elements Med Biol 12:231–235, 1998.
17. Pineda O, Ashmead HD, Perez JM, Lemus CP: Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. J Appl Nutr 46:2–13, 1994.
18. Cromer BA, Steinberg K, Gardner L, Thornton D, Shannon B: Psychosocial determinants of compliance in adolescents with iron deficiency. Am J Dis Child 143:55–58, 1989.
19. Wong BSM, Norman DC: Evaluation of a novel medication aid, the calendar blister-pak, and its effect on drug compliance in a geriatric outpatient clinic. J Am Geriatr Soc 35:21–26, 1987.
20. Gabriel M, Gagnon JP, Bryan CK: Improved patient compliance through use of a daily drug reminder chart. Pub Health Brief 67:968–969, 1977.
21. Drabkin DL, Austin JH: Spectrophotometric studies: spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J Biol Chem 98:719–733, 1932.
22. Heath A-LM, Skeaff CM, Gibson RS: The relative validity of a computerized food frequency questionnaire for estimating intake of dietary iron and its absorption modifiers. Eur J Clin Nutr 54:592–599, 2000.
23. Rangan A, Blight G, Binns C: Haem iron content in Australian meats and fish. Proc Nutr Soc Aust 20:210, 1996.
24. Steel RGD, Torrie JH: "Principles and Procedures of Statistics: A Biometrical Approach." New York: McGraw-Hill Book Company, 1980.
25. Senn SJ, Auclair P: The graphical representation of clinical trials with particular reference to measurements over time. Stat Med 9:1287–1302, 1990.
26. Hulthén L, Lindstedt G, Lundberg P-A, Hallberg L: Effect of a mild infection on serum ferritin concentration—clinical and epidemiological implications. Eur J Clin Nutr 52:376–379, 1998.
27. Hobbs FDR, Kenkre JE, Carter YH, Thorpe GH, Holder RL: Reliability and feasibility of a near patient test for C-reactive protein in primary care. Brit J Gen Prac 46:395–400, 1996.
28. Gardner MJ, Altman DG: Estimation rather than hypothesis testing: confidence intervals rather than P values. In Gardner MJ, Altman DG (eds): "Statistics with Confidence." London: British Medical Journal, pp 6–19, 1989.
29. Burlingame BA, Milligan GC, Apimerika DE, Arthur JM: "The Concise New Zealand Food Composition Tables." Palmerston North, NZ: New Zealand Institute for Crop & Food Research, 1993.
30. Hallberg L, Hultén L, Gramatkovski E: Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? Am J Clin Nutr 66:347–356, 1997.
31. Cook JD: Clinical evaluation of iron deficiency. Sem Hematol 19:6–18, 1982.
32. Hallberg L, Rossander L: Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American–type of meal. Am J Clin Nutr 39:577–583, 1984.
33. Layrisse M, Martínez-Torres C, González M: Measurement of the total daily dietary iron absorption by the extrinsic tag model. Am J Clin Nutr 27:152–162, 1974.
34. Coplin M, Schuette S, Leichtmann G, Lashner B: Tolerability of iron: a comparison of bis-glycino iron II and ferrous sulfate. Clin Ther 13:606–612, 1991.
35. Solomons NW: Competitive interaction of iron and zinc in the diet: consequences for human nutrition. J Nutr 116:927–935, 1986.
36. Skikne BS, Flowers CH, Cook JD: Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 75:1870–1876, 1990.
37. "Assessment of the Iron Nutritional Status of the U.S. Population Based on Data Collected in the Second National Health and Nutrition Examination Survey, 1976–1980." Bethesda: Life Sciences Research Office, Federation of American Societies for Experimental Biology, 1984.
38. Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD: Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. J Lab Clin Med 19:385–390, 1992.
39. Flowers CH, Skikne BS, Covell AM, Cook JD: The clinical measurement of serum transferrin receptor. J Lab Clin Med 114: 368–377, 1989.
40. Zhu YI, Haas JD: Response of serum transferrin receptor to iron supplementation in iron-depleted, nonanemic women. Am J Clin Nutr 67:271–275, 1998.

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