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ORIGINAL CONTRIBUTION

Suboptimal iron status and associated dietary patterns and practices in premenopausal women living in Auckland, New Zealand

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Abstract
Purpose To investigate associations between dietary patterns and suboptimal iron status in premenopausal women living in Auckland, New Zealand.
Methods Premenopausal women ($n = 375$; 18–44 years) were included in this cross-sectional analysis. Suboptimal iron status was defined as serum ferritin <20 μg/L. Participants completed a 144-item iron food frequency questionnaire (FeFFQ) and a questionnaire on dietary practices to assess dietary intake over the past month. Factor analysis was used to determine dietary patterns from the FeFFQ. Logistic regression was used to determine associations between these dietary patterns and iron status.
Results Seven dietary patterns were identified: refined carbohydrate and fat; Asian; healthy snacks; meat and vegetable; high tea and coffee; bread and crackers; and milk and yoghurt. Logistic regression suggested that following a "meat and vegetable" dietary pattern reduced the risk of suboptimal iron status by 41 % (95 % CI: 18, 58 %; $P = 0.002$) and following a "milk and yoghurt" pattern increased the risk of suboptimal iron status by 50 % (95 % CI: 15, 96 %; $P = 0.003$).
Conclusions These results suggest that dietary patterns characterized by either a low intake of meat and vegetables or a high intake of milk and yoghurt are associated with an increased risk of suboptimal iron status. Dietary pattern analysis is a novel and potentially powerful tool for investigating the relationship between diet and iron status.

Keywords Iron · Dietary patterns · Factor analysis · Women

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Introduction

Iron deficiency is common in developed and developing countries [1] and is associated with a number of health effects including reduced physical performance, deficits in cognitive function, and poor pregnancy outcome [2]. Young premenopausal women living in New Zealand are at risk of iron deficiency [3], and for some this is due to diet [4].

Iron bioavailability is more important than iron content in determining the amount of iron absorbed from a meal [5]. Ascorbic acid [6] and an as yet unidentified factor in meat, fish, and poultry enhance [7], while phytic acid, polyphenols, and calcium inhibit non-heme iron absorption [8–10]. Several cross-sectional studies that have included premenopausal women have investigated the association between individual nutrients (iron, ascorbic acid, and calcium) or foods and beverages (meat, fruit, vegetables, dairy products, tea, and coffee) and iron status [4, 11–15]. However, the results have been somewhat inconsistent. For example, in most [4, 13–15] but not all [11, 12] cross-sectional studies, meat consumption has been associated with an increased iron status. An inverse association between calcium intake and iron status [13, 14] has been demonstrated in some studies, while other studies have observed no effect [4, 12, 15].

The focus on individual nutrients and foods in cross-sectional studies has several limitations, as described by Hu [16] and Newby et al. [17], a number of which are relevant to iron nutrition. People do not eat nutrients alone but consume meals containing many combinations of foods and nutrients that may interact [16, 17]—for example, non-heme iron is influenced by enhancers and inhibitors of iron absorption consumed in the same meal. Statistically significant associations may occur by chance when several foods and nutrients are analyzed separately [16], and single nutrient analysis may be confounded by the effect of dietary patterns [16]. For example, although phytic acid in whole grains inhibits iron absorption, this effect may be negated or blunted if sufficient ascorbic acid is consumed at the same time [18], for instance if whole grains are consumed with fruits and vegetables high in ascorbic acid.

Dietary pattern analysis using factor or cluster analysis offers an alternative approach to studying individual foods and nutrients and their association with iron status [16, 17]. Only two studies have explored the association between dietary patterns and iron status [19, 20]. In China, "traditional" and "sweet tooth" dietary patterns were positively associated, and a "healthy" dietary pattern was negatively associated with anemia [19]. In Norway, high serum ferritin concentrations were associated with a "reindeer meat" dietary pattern [20].

Dietary pattern analysis describes combinations of foods consumed in the diet as a whole. However, factors impacting on iron bioavailability are more likely to affect non-heme iron absorption if consumed at the same time [21, 22], so it is also important to investigate dietary practices such as the regular consumption of particular foods together in the same meal. One study found women ($n = 15$) with a serum ferritin <12 μg/L had a higher intake of tea and lower intake of ascorbic acid at meals [23], suggesting that dietary practices may impact on iron status.

Combinations of foods and beverages may be more strongly related to iron status than individual foods and nutrients. This study aimed to investigate the dietary patterns and practices of premenopausal women living in New Zealand in relation to their risk of suboptimal iron status.

Methods

Study design and participants

This cross-sectional study was undertaken in Auckland, New Zealand, between March and September 2008. Participants visited the Human Nutrition Research Unit (HNRU) at Massey University's Albany campus on one occasion during which demographic data (age, ethnicity) were collected, anthropometric measurements were made, and dietary intake was assessed.

A total of 404 women, aged between 18 and 44, who were participating in two separate studies were included in this analysis: one investigating iron status in female university students ($n = 276$) [24] and the other screening women from the general population for participation in a randomized controlled trial investigating the effect of a dietary intervention on iron status ($n = 128$) [25]. For the analysis conducted in this study, exclusion criteria were: current pregnancy, pregnancy in the past year, current breastfeeding; any known health problems likely to influence iron status including inflammatory bowel disease, celiac disease; and history of gastric ulcers, red blood cell disorders, menorrhagia, hemorrhoids, hematuria, or malaria. Women who regularly consumed iron supplements (20 mg elemental iron or more at least 3–4 times/week) within the 3-month period prior to the study were also excluded from the analysis.

The procedures followed were in accordance with the ethical standards of the Massey University Human Ethics Committee: (Southern A), Reference No 07/73 and 08/20, and all participants gave written informed consent.

Blood sampling and biochemical analysis to determine iron status

A venipuncture blood sample was taken at the HNRU or at Diagnostic MedLab, Auckland between 7 am and 5 pm. Four milliliters of blood was collected into an EDTA tube for analysis of hemoglobin, and 3.5 mL of blood was collected into a SST (serum separator tube) for the analysis of serum ferritin (SF) and C-reactive protein (CRP). Hemoglobin, SF, and CRP were analyzed at Diagnostic MedLab, an International Accreditation New Zealand laboratory. Serum ferritin was analyzed using the immunoturbidimetric test (Roche Diagnostics, Indianapolis) (Cat. No. 11661400) and CRP using the particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Indianapolis) (Cat. No. 03002039). Hemoglobin was analyzed using the SLS-Hb (sodium lauryl sulfate-Hb) method using an automated hematology analyser XE-2100 (Sysmex Corporation, Auckland, NZ).

Participants were divided into those with sufficient iron stores (SF ≥ 20 μg/L and hemoglobin ≥ 120 g/L) versus participants with suboptimal iron status (SF < 20 μg/L and hemoglobin < or ≥ 120 g/L) [26]. Participants who had anemia without iron deficiency (SF ≥ 20 μg/L, hemoglobin < 120 g/L) were excluded from the analysis.

Anthropometric measurements

Height and weight were measured in duplicate by a trained researcher using the International Society for the Advancement of Kinanthropometry protocols [27]. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$.

Dietary assessment

Participants completed two questionnaires to assess dietary intake: an iron food frequency questionnaire (FeFFQ) to determine dietary patterns, and a questionnaire to assess dietary practices. The FeFFQ was developed to assess intake of foods containing iron or affecting iron bioavailability. The dietary practices questionnaire considered the various combinations in which foods and beverages were consumed.

Determination of dietary patterns

The FeFFQ considered food consumption over the past month. It collected information on frequency of consumption (9 options) for 144 food groupings. Portion size was not specified within the FeFFQ. An extensive list of food groupings was developed based on the foods in the New Zealand 1997 National Nutrition Survey food frequency questionnaire [28] and food composition data from the New Zealand Food Composition Tables [29]. Foods were grouped according to their similarities (e.g., stone fruits were grouped together), frequency of consumption (e.g., bananas were kept as a separate item because they are consumed frequently [28]) and nutrient content of iron, ascorbic acid, and calcium per common standard measure [29]. These nutrients were chosen due to their potential impact on iron intake and bioavailability (e.g., red cabbage was grouped separately to other cabbages due to its higher ascorbic acid and calcium content).

The final FeFFQ contained 144 food groupings (each corresponding to a single question) categorized into 16 food categories: meat/chicken, prepared meat, fish/seafood, eggs, nuts, legumes, dairy products, fruit, vegetables, breakfast cereals/porridge, grains/cereals, breads, cakes/biscuits/crackers, miscellaneous foods/drinks, alcoholic beverages, and non-alcoholic beverages.

Responses from the FeFFQ were converted into nine frequencies of intake per week for each food grouping for each participant, ranging from zero to 28 times eaten per week. The average weekly consumption of each food grouping was determined, and the 30 most frequently consumed food groupings (as number of times consumed per week) were identified for use in the factor analysis. The 30 most frequently consumed food groupings were selected as they were consumed at least two times per week on average. Overall, they accounted for 61.8 % of the total number of food groupings from the FeFFQ consumed per week.

Determination of dietary practices

A separate questionnaire was developed to investigate dietary practices and to determine whether particular combinations of foods and beverages consumed at main meals and snacks affected iron status. In the dietary practices questionnaire, participants were given a list of foods: breakfast cereals, porridge, bread/toast, noodles/rice, milk products, fruit, vegetables, meats, baked beans/eggs, starchy foods (e.g., pasta, rice, potato), legumes, nuts, and eggs and asked to indicate which foods were a part of their usual breakfast, lunch, and evening meals. Beverages consumed with these meals or within 1 h of a meal or snack were also investigated: fruit/vegetable juice, milk, soy or chocolate-based drinks, coffee, and tea. Habitual food and beverage intake between meals were investigated and in addition to the food items listed above, typical snack choices were investigated: biscuits/cakes, crackers, potato crisps, cereal/muesli bars, and chocolate/sweets.

Statistical analysis

Statistical analysis was performed using Predictive Analytics SoftWare (PASW) Statistics 18 [30]. Two-sided tests were used for all analyses.

Determination of dietary patterns from the FeFFQ using factor analysis

Dietary patterns from the FeFFQ were identified using factor analysis [31]. Factor analysis aggregates food groupings based on the degree to which food groupings in the dataset are correlated with one another [17]. Principal components analysis and the orthogonal varimax rotation were used to facilitate interpretability of factors. The Kaiser–Meyer–Olkin measure of sampling adequacy was 0.607 (>0.5 acceptable), and Bartlett's Test P value was <0.001 (<0.05 acceptable), demonstrating the presence of relationships between variables in the factor analysis.

To decide which factors to retain, factor solutions ranging from two to 10 were run. Factors with an eigenvalue >1 were considered. Both the scree plot and the factors themselves were examined to see which factors had the most meaningful dietary patterns. Labeling of dietary patterns was based on the interpretation of foods with high factor loadings for each dietary pattern (equivalent to simple correlations between the food groupings and dietary patterns). Only foods positively associated with a factor loading of ≥0.3 were included.

Seven factors were retained and considered as the major dietary patterns of these participants. A dietary pattern score was created for each individual for each dietary pattern. The regression method in PASW was used to estimate these scores. These dietary pattern scores were used in subsequent statistical analysis to examine the relationship between dietary patterns and iron status.

Dietary patterns and suboptimal iron status

Participants were divided into those with sufficient iron stores versus participants with suboptimal iron status. It was calculated, retrospectively, using G*Power 3 [32] that a sample size of 375 (final sample used in the analysis) provided 87 and 99 % power to detect significant differences ($\alpha = 0.05$) and odds ratios (OR) of 1.5 and 0.5, respectively, between participants with sufficient iron stores and suboptimal iron status. Dietary pattern scores were divided into quintiles. Non-normally distributed variables were transformed into approximately normal distributions by logarithmic transformations and again tested for normality. Differences between groups were tested using the independent *t* test for parametric data, the Mann–Whitney test for non-parametric data, and the chi-square test to investigate categorical variables.

Stepwise multiple logistic regression analysis was performed to determine which dietary patterns contributed independently to suboptimal iron status (dependent variable) while considering the effects of age, BMI, ethnicity (European versus Asian; European versus other), and other dietary patterns. Dietary patterns, age, and BMI were entered into the model as continuous variables and ethnicity as a categorical variable. The entry criterion was set at $P < 0.05$ for forward stepwise multiple logistic regression analysis. All assumptions for multicollinearity were met.

Dietary practices and suboptimal iron status

Chi-square analysis was used to investigate dietary practices, including foods and beverages consumed at main meals and snacks for participants who had sufficient iron stores versus participants with suboptimal iron status. For participants consuming potential sources of iron at main meals (e.g., meat), we investigated whether foods or beverages affecting iron bioavailability were consumed at the same time (e.g., milk products). The *P* value for analyzing dietary practices was set at $P < 0.01$ due to the multiple comparisons that were made.

Results

Iron status and characteristics of participants

Of the 404 participants who took part in this study, data for 29 were excluded: blood results were unavailable for three participants, four did not complete the dietary questionnaires, four participants with a CRP > 10 mg/L [33] (marker of inflammation) were removed from the analysis as serum ferritin is an acute phase protein and is artificially increased during infection or inflammation [34], 16 participants had anemia without iron deficiency (SF ≥ 20 μg/L, hemoglobin < 120 g/L), and two participants with SF > 200 μg/L [35] were excluded from the analysis due to possible associations with hemochromatosis [36]. Of the remaining 375 participants included in the analysis, 70 (18.7 %) had suboptimal iron status (SF < 20 μg/L and hemoglobin < or ≥ 120 g/L). Of these, 20 (28.6 %) had iron deficiency anemia (SF < 20 μg/L, hemoglobin < 120 g/L). Table 1 shows the characteristics of the study participants. Participants with suboptimal iron status were more likely to be older ($P = 0.033$) and of Asian ethnicity ($P = 0.002$).

Dietary patterns

The 30 most frequently consumed food groupings are listed in Table 2 with the factor loadings for each dietary pattern (factor loadings of 0.3 or higher are in bold). Seven dietary patterns were determined using factor analysis: refined carbohydrate and fat, Asian, healthy snacks, meat and

Table 1 Characteristics of study participants with and without suboptimal iron status

Characteristics	Participants with sufficient iron stores[a] ($n = 305$)	Participants with suboptimal iron status[b] ($n = 70$)	*P* value for difference
Age (years)	25 (20, 35)	29 (21, 40)	0.033
BMI (kg/m^2)	22.7 (20.8, 24.9)	22.3 (21.1, 24.4)	0.711
SF (μg/L)	46 (34, 63)	13 (9, 17)	<0.001
Hemoglobin (g/L)	133 (128, 139)	126 (119, 132)	<0.001
Ethnicity n (%)			0.002
European	239 (78.6)	43 (61.4)	
Asian	36 (11.8)	20 (28.6)	
Other	29 (9.5)	7 (10.0)	

All results expressed as median (25, 75 percentile) or n (%)

SF serum ferritin (μg/L)

[a] SF ≥ 20 μg/L and hemoglobin ≥ 120 g/L

[b] SF < 20 μg/L and hemoglobin < 120 or ≥ 120 g/L

Table 2 Factor loadings for each food grouping for the seven dietary patterns identified

Food groupings as contained in the FeFFQ	Factor components and factor loadings							Frequency of consumption per week[a]
	DP 1: refined carbohydrate and fat	DP 2: Asian	DP 3: healthy snacks	DP 4: meat and vegetables	DP 5: high tea and coffee	DP 6: bread and crackers	DP 7: milk and yoghurt	
Butter or margarine	**0.62**	−0.10	−0.09	−0.05	0.23	**0.33**	−0.15	5.1 (4.6, 5.7)
Potatoes	**0.59**	0.02	−0.06	0.25	−0.12	0.01	0.26	2.5 (2.3, 2.7)
Jam	**0.52**	−0.04	0.20	−0.06	0.06	0.04	−0.09	2.2 (1.9, 2.6)
White bread and rolls	**0.52**	0.09	−0.26	−0.05	−0.05	0.19	−0.08	2.3 (2.0, 2.7)
Sugar	**0.51**	0.14	−0.06	0.02	0.09	−0.06	0.24	7.7 (6.9, 8.6)
Onions, leeks, celery	−0.01	**0.80**	0.00	0.10	0.08	−0.05	−0.03	3.3 (3.0, 3.6)
Tomatoes	−0.07	**0.77**	0.11	−0.05	0.06	0.24	0.13	3.4 (3.0, 3.7)
Cooking oil	**0.44**	**0.64**	0.02	0.00	−0.10	−0.18	−0.02	5.3 (4.9, 5.6)
Apples	−0.14	0.02	**0.68**	0.04	−0.05	−0.05	0.01	3.2 (2.9, 3.5)
Bananas	0.04	0.04	**0.58**	0.12	0.05	0.06	0.15	3.1 (2.8, 3.4)
Citrus fruits	0.13	0.15	**0.55**	−0.07	−0.22	−0.10	−0.03	2.8 (2.5, 3.1)
Herbal tea, fruit tea	−0.09	−0.06	**0.39**	−0.03	0.10	0.09	−0.16	2.7 (2.2, 3.2)
Chicken, turkey or duck	0.18	−0.09	−0.09	**0.64**	0.04	−0.17	−0.07	2.2 (2.0, 2.3)
Broccoli	−0.09	0.03	0.05	**0.63**	−0.05	0.10	0.13	2.2 (2.0, 2.4)
Carrots	−0.01	0.16	0.28	**0.54**	0.12	0.04	0.08	3.3 (3.0, 3.6)
Capsicum, peppers	−0.33	**0.36**	0.04	**0.46**	0.13	0.20	−0.05	2.0 (1.8, 2.2)
Lettuce	−0.14	**0.30**	0.06	**0.45**	−0.09	**0.45**	−0.04	3.1 (2.8, 3.5)
Beef	0.28	−0.22	−0.12	**0.41**	−0.00	−0.16	−0.09	2.0 (1.8, 2.2)
Milk (cows milk) added to drinks	0.21	0.03	−0.10	0.04	**0.77**	0.08	0.15	11.0 (10.0, 11.9)
Coffee	0.05	0.08	0.01	0.04	**0.65**	0.06	0.02	6.7 (5.9, 7.6)
Black tea	0.03	−0.01	0.14	−0.09	**0.55**	−0.02	−0.23	2.7 (2.1, 3.3)
Fruit and vegetable juices	0.13	0.02	0.03	−0.05	−0.29	0.05	−0.09	2.0 (1.7, 2.2)
White rice	0.14	**0.32**	0.07	0.02	−0.05	−0.64	−0.19	2.5 (2.1, 2.8)
Cheese	0.28	0.16	−0.05	−0.07	−0.01	**0.59**	0.01	3.0 (2.7, 3.3)
Brown bread and rolls	0.24	0.03	**0.38**	0.00	0.13	**0.46**	−0.18	5.4 (4.8, 5.9)
Crackers	0.21	0.29	0.15	0.13	−0.10	**0.38**	−0.11	2.2 (1.9, 2.5)
Milk (cows milk) added to food	0.05	−0.10	0.01	−0.02	0.25	0.12	**0.69**	4.5 (4.0, 4.9)
Milk (cows milk) as a drink	0.16	0.03	−0.13	0.02	−0.18	−0.20	**0.52**	2.0 (1.6, 2.4)
Yoghurt	−0.19	0.11	**0.35**	0.03	0.06	0.04	**0.43**	3.0 (2.7, 3.3)
Water	−0.17	0.11	0.23	0.17	−0.20	0.12	0.25	21.8 (20.9, 22.6)
Variance in the intake scores (%)	7.9	7.5	6.3	6.2	5.9	5.9	4.7	

FeFFQ iron food frequency questionnaire, *DP* dietary pattern

Factor loadings >0.3 are presented in bold

[a] Frequency of consumption reported as means (95 % CI)

vegetables, high tea and coffee, bread and crackers; and milk and yoghurt. The seven patterns identified explained 44.3 % of the variance in the intake scores.

Dietary patterns and iron status

Table 3 compares the characteristics of participants with dietary pattern scores in the highest (quintile five) and lowest (quintile one) quintiles for each dietary pattern. Participants in the highest quintile for the "meat and vegetable" dietary pattern had significantly higher serum ferritin and hemoglobin concentrations and were less likely to have suboptimal iron status than those in quintile one. In contrast, participants in the highest quintile for the "milk and yoghurt" dietary pattern had a significantly lower serum ferritin and greater levels of suboptimal iron status than those in quintile one. Two other patterns were also significantly associated with iron status. Participants in the highest quintile for the "high tea and coffee" dietary pattern had significantly lower serum ferritin concentrations.

Table 3 Characteristics of participants with dietary pattern scores in the lowest (quintile 1) and in the highest (quintile 5) quintiles for the seven dietary patterns

	DP 1: refined carbohydrate and fat		DP 2: Asian		DP 3: healthy snacks		DP 4: meat and vegetables	
	Quintile 1	Quintile 5	Quintile 1	Quintile 5	Quintile 1	Quintile 5	Quintile 1	Quintile 5
Age (years)	26 (21, 35)	25 (20, 37)	**22 (19, 32)**	**26 (21, 33)***	**22 (19, 31)**	**27 (21, 37)***	25 (20, 34)	25 (20, 35)
BMI (kg/m^2)	22 (21, 24)	23 (21, 27)	23 (21, 25)	23 (20, 26)	23 (20, 25)	22 (21, 25)	23 (21, 24)	23 (21, 27)
SF (µg/L)	40 (28, 51)	36 (21, 57)	36 (21, 57)	43 (26, 60)	34 (18, 58)	38 (21, 47)	**29 (18, 42)**	**48 (37, 64)*****
Hemoglobin (g/L)	132 (127, 138)	132 (126, 137)	132 (127, 139)	133 (126, 138)	131 (125, 137)	130 (126, 137)	**129 (123, 133)**	**133 (128, 140)*****
Suboptimal iron status (%)	11 (14.7)	16 (21.3)	16 (21.3)	14 (18.7)	19 (25.3)	16 (21.3)	**21 (28)**	**5 (6.7)*****
Ethnicity n (%)								
European	58 (78.4)	51 (68.0)	59 (79.7)	**42 (56.0)*****	58 (77.3)	47 (63.5)	54 (72)	61 (81.3)
Asian	8 (10.8)	16 (21.3)	8 (10.8)	**26 (34.7)**	8 (10.7)	16 (21.6)	16 (21.3)	7 (9.3)
Other	8 (10.8)	8 (10.7)	7 (9.5)	**7 (9.3)**	9 (12.0)	11 (14.9)	5 (6.7)	7 (9.3)

	DP 5: high tea and coffee		DP 6: bread and crackers		DP 7: milk and yoghurt	
	Quintile 1	Quintile 5	Quintile 1	Quintile 5	Quintile 1	Quintile 5
Age (years)	**22 (19, 30)**	**34 (26, 40)*****	26 (21, 34)	26 (20, 38)	**28 (22, 39)**	**22 (19, 32)****
BMI (kg/m^2)	22 (21, 24)	23 (22, 26)	23 (20, 24)	23 (21, 27)	23 (21, 25)	22 (20, 24)
SF (µg/L)	**44 (32, 74)**	**38 (19, 58)***	39 (18, 63)	41 (29, 58)	**43 (29, 62)**	**33 (17, 53)***
Hemoglobin (g/L)	132 (127, 137)	131 (126, 137)	131 (125, 137)	132 (127, 138)	132 (126, 139)	131 (125, 136)
Suboptimal iron status (%)	11 (14.7)	19 (25.3)	**20 (26.7)**	**8 (10.7)***	**9 (12.0)**	**22 (29.3)***
Ethnicity n (%)						
European	57 (76)	61 (81.3)	**29 (39.2)**	**62 (82.7)*****	**51 (68.0)**	**55 (74.3)***
Asian	11 (14.7)	9 (12.0)	**36 (48.6)**	**3 (4.0)**	**18 (24.0)**	**15 (20.3)**
Other	7 (9.3)	5 (6.7)	**9 (12.2)**	**10 (13.3)**	**6 (8.0)**	**4 (5.4)**

Results expressed as either median (25, 75 percentile) or n (%); for each quintile, $n = 75$

Difference between groups (independent *t* test, Mann–Whitney test or chi-square test)

Statistically significant differences between quintile 1 and 5 are presented in bold

DP dietary pattern; *SF* serum ferritin (µg/L)

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

In contrast, participants in the highest quintile for the "bread and crackers" dietary pattern were less likely to have suboptimal iron status than participants in quintile one. There were no significant differences in iron status between quintiles one and five for the "refined carbohydrate and fat," "healthy snacks," and "Asian" dietary patterns.

When all seven dietary patterns, age, ethnicity, and BMI were entered into the stepwise multiple logistic regression analysis, it was found that participants following the "meat and vegetable" dietary pattern reduced their risk of suboptimal iron status by 41 % ($P = 0.002$) and following a "milk and yoghurt" dietary pattern increased the risk of suboptimal iron status by 50 % ($P = 0.003$) (Table 4). Being of Asian ethnicity almost tripled the risk of suboptimal iron status ($P = 0.002$), while the risk of suboptimal iron status increased slightly with each year of age ($P = 0.003$).

The results did not change when the variables that were excluded from the original stepwise multiple regression model due to non-significance (dietary patterns "refined carbohydrate and fat," "Asian," "healthy snacks," "high tea and coffee," and "bread and crackers"; BMI; other ethnicity) and were added independently to the basic model (including the "meat and vegetable" dietary pattern, "milk and yoghurt" dietary pattern, Asian ethnicity, and age) using forced entry multiple logistic regression analysis. There was no evidence of an interaction effect between the "meat and vegetable" and "milk and yoghurt" dietary patterns. Furthermore, the percentage of participants who scored low (quintile one) versus high (quintile five) on the "milk and yoghurt" pattern did not differ according to which quintile they belonged to on the "meat and vegetable" pattern ($P = 0.473$), suggesting that participants who consumed higher amounts of milk and yoghurt were doing so independently of their intake of meat and vegetables (data not shown).

Table 4 Multiple logistic regression model for associations between dietary patterns and suboptimal iron status

	Exp(B)[a]	95.0 % CI for exp (B)		*P* value
		Lower	Upper	
Dietary pattern 4 meat and vegetables	0.589	0.422	0.820	0.002
Dietary pattern 7. milk and yoghurt	1.502	1.153	1.957	0.003
Asian ethnicity	2.961	1.509	5.811	0.002
Age (years)	1.051	1.017	1.086	0.003

Variables included in the model: age, ethnicity (European versus Asian; European versus other), BMI, and all seven dietary patterns

$R^2 = 0.104$ (Hosmer and Lemeshow), 0.095 (Cox and Snell), 0.154 (Nagelkerke), Model $\chi^2 = 37.50$

[a] Change in the odds of suboptimal iron status occurring for each unit change in the predictor variable. If >1.0, as predictor variable increases, odds of sub optimal iron status increase. If <1.0, as predictor variable increases, odds of suboptimal iron status decreases

Dietary practices and iron status

Analysis of the dietary practices revealed that participants with sufficient iron stores were more likely to consume milk or milk products between meals or at supper time (52.0 %) than were participants with suboptimal iron status (32.9 %) ($P = 0.005$). There were no other significant differences in dietary practices identified between participants with sufficient versus suboptimal iron status.

Discussion

Seven dietary patterns were identified in this population of healthy premenopausal New Zealand women. A "meat and vegetable" dietary pattern was associated with a lower risk while a "milk and yoghurt" dietary pattern was associated with an increased risk of suboptimal iron status.

The "meat and vegetable" pattern consisted of beef, chicken, capsicum, broccoli, carrots, and lettuce. Meat contains both heme and non-heme iron. Heme iron is better absorbed than non-heme iron, and its absorption is less likely to be affected by a person's iron status and other dietary factors [37, 38]. In addition, beef and chicken contain the meat/fish/poultry factor (MFP factor) which enhances non-heme iron absorption [7]. Most cross-sectional studies have observed an increased iron status with a higher meat intake [4, 13–15], although some have found no association [11, 12]. In Norway, Broderstad et al. [20] found high serum ferritin concentrations to be associated with a "reindeer meat" dietary pattern. No association was found between a dietary pattern consisting of meat and alcohol in Chinese adults; however, the average daily meat consumption was low, and all those with anemia did not necessarily have iron deficiency anemia [19]. Capsicum and broccoli are good sources of ascorbic acid [29], which enhances non-heme iron absorption [6].

Participants following a "milk and yoghurt" dietary pattern (milk added to food, milk as a drink, and yoghurt) had an increased risk of suboptimal iron status. Both milk and yoghurt are high in calcium, which inhibits both heme and non-heme iron absorption [39]. However, calcium's effect is less clear and weaker than that of other inhibitors of iron absorption. While some cross-sectional studies in premenopausal women have found no association between dairy product or calcium intake and iron status [4, 12, 15], others have found a negative association [13, 14, 40]. A dietary pattern consisting of drinks, milk, and cake was positively associated with anemia in Chinese adults [19]. However, long-term calcium supplementation does not appear to affect iron status in healthy female adolescents [41] or adults [42]. Statistical analysis suggested that the "milk and yoghurt" dietary pattern exerted an independent effect on iron status, which was not due to the replacement of meat and vegetables with milk and yoghurt, as has been suggested previously [4]. This was confirmed by a post hoc analysis which showed the effect of the "milk and yoghurt" dietary pattern was still significant after controlling for frequency of meat, poultry, and fish intake entered as a single continuous variable. However, it was not possible to determine whether there were differences in the absolute intake of meat associated with the "milk and yoghurt" dietary pattern as serving size information was not collected.

Analysis of dietary practices suggested that participants with sufficient iron stores were more likely to consume milk or milk products between meals than participants with suboptimal iron status. However, the consumption of other foods and beverages in various combinations at meals and in between meals was not associated with iron status, including consumption of milk and milk products with meals. The only study that has observed a difference in iron status when investigating consumption of beverages in relation to meals reported an increased risk when tea was consumed with main meals. However, this study was undertaken in 15 institutionalized mentally handicapped women [23], whose diets are likely to be more regulated than those of women living independently. In practice, women consume a variety of foods in a variety of combinations across the day. The dietary practices questionnaire was not validated meaning we cannot be sure it measured what it was intended to measure. Furthermore, it may not have been sensitive enough and our sample size may have been too small to account for the effects of all possible combinations of foods eaten. For these reasons, it is difficult to reach a conclusion about how dietary practices may impact on iron status.

This is the first study, to our knowledge, to focus on dietary patterns and iron status in premenopausal women using at least two measures of iron status. Serum ferritin allowed us to determine whether iron deficiency was present and hemoglobin indicated the presence of anemia [26]. In contrast, previous studies investigating the relationship between dietary patterns and iron status did not use hemoglobin and serum ferritin in combination [19, 20]. As a result, the severity of iron deficiency is not known in one study [20] and the etiology of the anemia in the other study is unknown and is not necessarily iron deficiency [19].

We used the 30 most frequently consumed foods from the FeFFQ in the factor analysis. Food groupings were considered likely to have an impact on iron status only if they were consumed at least twice per week. It has been suggested that for most people, consuming 15–20 different foods per week should be achievable and that optimal health occurs when 30 or more biologically distinct foods are consumed per week [43]. As our FeFFQ was not restricted in the development phase to commonly consumed foods, some foods were consumed infrequently (e.g., black pudding was only consumed on average 0.01 times per week) and were therefore likely to have minimal effects on iron status so were not included in the factor analysis. Foods that may contribute positively to iron status that did not appear in the 30 most frequently consumed foods included fish (consumed 1.7 times per week after combining the three categories of fish within the FeFFQ) and breakfast cereal (consumed 1.4 (muesli) to 0.1 times per week (chocolate-based cereals)). Of studies that have investigated the independent effect of fish on iron status [14, 20, 44], only one has observed a positive relationship between fish consumption and serum ferritin concentrations [14]. Cade et al. [45] found no association between serum ferritin concentrations and intake of fortified breakfast cereals.

In a separate study, the validity and reproducibility of the FeFFQ were investigated in 115 women, aged between 18 and 44, using a weighed four-day food record and a FeFFQ completed on two occasions, 1 month apart. The FeFFQ demonstrated good validity (compared to the weighed record) and high reproducibility (compared to the second administration of the FeFFQ) for the frequency of intake of food groupings. The majority of food groupings were comparable, with only three out of 30 foods showing meaningful differences (i.e., medium effect sizes: $r > 0.3$ [31]) between the FeFFQ and food record. The average Spearman rank correlation coefficient was 0.52 and ranged from 0.27 to 0.89. High reproducibility was seen between the two administrations of the FeFFQ, with an average Spearman rank correlation coefficient of 0.76 (range: 0.50–0.93), and a comparable frequency of intake (all effect sizes were small: $r < 0.3$ [31]).

The use of factor analysis to determine dietary patterns overcomes some of the limitations inherent in using food composition databases to assess nutrient intake [26], such as the natural variation in food composition, limited coverage of food items (requiring substitutions) or nutrients (e.g., phytic acid data are not available in the New Zealand Food Composition database), and inappropriate food composition values within the database due to random and systematic errors [26]. The use of factor analysis to determine dietary patterns does, however, involve several subjective decisions [16, 17]. These include the number of food groups to enter into the factor analysis, the number of factors to extract, and interpretation of the results, including factor loadings and labeling dietary patterns [16, 17].

In conclusion, a "meat and vegetable" dietary pattern was associated with lower and a "milk and yoghurt" dietary pattern with a higher risk of suboptimal iron status independent of age, ethnicity, or other dietary patterns in this group of healthy premenopausal women. These findings reinforce our knowledge of meat's beneficial effect on iron status and provide some support for calcium (or another component in milk and yoghurt) having a negative effect on iron status in the context of the whole diet. Further research is needed to explore the relationship between iron status and intake of dairy products, with a focus on how and when these are consumed. However, as causality cannot be established in a cross-sectional study design, it is not known whether these dietary patterns caused suboptimal iron status, or whether women with suboptimal iron status were more likely to consume these dietary patterns. The use of dietary patterns to determine predictors of suboptimal iron status confirms that both enhancers and inhibitors of iron absorption influence iron status. Analysis of dietary patterns is an effective way to assess the impact of diet on iron status and potentially provides more relevant information than individual nutrients or foods alone.

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Conflict of interest The authors declare that they have no conflict of interest.

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