Factors affecting iron status in 15-30 year old female students

AM Rangan[1] Bsc, GradDipNutrDiet, I Aitkin[2] PhD, GD Blight[2] BMedSc, MBBS, PhD, CW Binns[2] MBBS, MPH, FRACGP, FACOM, FAFPHM

[1] *NSW Breast Cancer Institute, Westmead, NSW, Australia*
[2] *School of Public Health, Curtin University of Technology, Perth, WA, Australia*

Limited Australian data are available on iron status and factors affecting iron status in young women. Iron status was assessed in a population of 15-30 year old students using standard haematological and biochemical tests. Data were collected on demographic details and known risk factors for iron deficiency, including diet. Iron deficiency was present in 7.2% and iron deficiency anaemia in 4.5% of this population, comparable to previously published Australian data. Using logistic regression, the factors found to be associated with low iron stores (serum ferritin <20 μg/L) included high social status, low haem iron intake, high calcium intake, a high menstrual score and a recent history of blood donation in women with BMI<24. Of these factors, increasing haem iron consumption (meat, chicken and fish) is the most appropriate and easily modifiable factor for public health intervention in this age group.

Key words: iron, haemoglobin, anaemia, women, Australia, food intake, vegetarian, vitamin C density, calcium intake, social status, menstrual function, blood donation, BMI (body mass index)

Introduction

Iron deficiency is reported to be the most common nutritional deficiency in the world[1]. Australian surveys suggest that those at greatest risk of iron deficiency are adolescent girls[2], pregnant women[3], female blood donors[3] and vegetarians[4], with prevalence estimates of 9-10% in these groups. The prevalence of iron deficiency is not well defined in 15-30 year old women even though, due to increased iron requirements and a compromised intake, this is one of the most vulnerable subgroups at risk.

Iron requirements are increased in adolescent girls with growth and the onset of menarche and remain high in women until menopause. Iron intake, on the other hand, is often compromised due to altered eating habits which may occur as a result of voluntary restriction of food intake, a change in living conditions, poor nutrition or cooking knowledge. National surveys report that 13-40% of Australian women fail to meet 70% of the recommended dietary intake (RDI) for iron[5] (the lower limit of the RDI for iron is 10 mg for 15-18 year old females and 12 mg for 19-30 year old females[6]). Haem iron intake may be compromised as a result of decreasing red meat consumption and an increasing trend towards vegetarianism. Other dietary factors, such as vitamin C and calcium are also known to affect iron absorption[7].

Apart from diet and a history of blood donation, there are several additional factors which may influence iron status in young women; use of the oral contraceptive pill (OCP) and intrauterine devices (IUD), strenuous exercise, and heavy alcohol intake[5,8]. The objectives of this study were to determine the iron status of 15-30 year old female students and to identify factors associated with iron deficiency in this population.

Methodology

Subjects

A total of 265 female students, aged 15-30 years, participated in this cross-sectional study. Student volunteers were recruited from the campus of Curtin University of Technology, Perth, Australia (n=224) and from secondary schools in the Perth area (n=41). The study was approved by the Human Ethics Committee of Curtin University of Technology. Subjects were excluded from the study if they were pregnant, post-partum, lactating or had resided in Australia for less than one year. Height was measured without shoes to the nearest 0.5 cm. Weight, in light clothing, was measured to the nearest 0.5 kg. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2).

Students entering the study were asked to complete a questionnaire which provided details of age, social status, medical history and factors which could affect iron status, such as dietary habits and supplement use, oral contraceptive use, frequency and duration of menstruation, number of parturitions, blood donation, and exercise patterns. Social status was assessed using Daniel's scale, based on the prestige of the father's occupation[9]. A menstrual score combining frequency and duration of menstruation was constructed, with three categories based on number of menstrual days per year: low (< 52), medium (52-65) and high (>65). An exercise level of low, medium or high, as described in the National Health Survey was ascribed to each student who reported undertaking some exercise[10].

A quantitative food frequency questionnaire (FFQ) was administered consisting of 270 food and drink items[11] and was validated for this study population, using 7-day weighed food records. The FFQs were analysed using the NUTTAB91 database. Haem iron intake was calculated manually for each subject, using haem iron contents of Australian meats[12]. All under-reporters, based on Goldberg's criteria, and over-reporters (>15 000 kJ) were excluded from further analyses[13].

Correspondence address: Anna Rangan, NSW Breast Cancer Institute, PO Box 143, Westmead, NSW 2145, Australia
Tel: (02) 9893 7288; Fax: +612 9845 7246

Laboratory analysis

A non-fasting venous blood sample was taken to measure standard haematological [haemoglobin (Hb), mean cell volume (MCV), and erythrocyte sedimentation rate (ESR)] and biochemical parameters of iron status [serum iron (SI), serum transferrin (Tn), transferrin saturation (TS) and serum ferritin (SF)]. A Coulter Counter (Model STKS) was used to determine the haematological parameters. Serum iron was assayed colourimetrically using the guanidine/ferrozine method and was performed without deproteinisation[14]. Serum ferritin was assayed by immunoturbidometry using latex particles coated with antibodies to ferritin (Tina-quant®, Boehringer Mannheim). Transferrin was assayed by immunoturbidometry using goat anti-human-transferrin/ TRIS/ polyethylene glycol (Tina-quant®, Boehringer Mannheim). Transferrin saturation was calculated using the formula: serum iron/ (transferrin x 20) x 100.

Data analysis

Prior to statistical analyses, skewed distributions were log-transformed (serum ferritin). A retransformation back to original units was made before reporting the results. The 95% confidence interval of the mean (95% CI) and percentile ranges are reported for iron status parameters. Logistic regression was used to determine the factors predictive of low iron stores (serum ferritin <20 μg/L[15]) and to estimate the magnitude of the association between predictor variables and outcome by obtaining odds ratios. The nutrient variables were entered into the model as nutrient densities, together with energy intake. This model, termed the multivariate nutrient density model, has been described by Willet[16]. It controls for confounding by energy intake, allowing the coefficient for the nutrient density variable to represent the relation of the nutrient composition of the diet while holding total energy intake constant. All statistical analyses were computed using SPSS-Windows, Version 6.1, Chicago 1995.

Results

Descriptive data

The age range of the students was 15-30 years with a mean of 20.7 years (SD 3.5 years). Most students (55%) were classified in the healthy weight range (BMI 20.0-25.0), 32% were underweight (BMI < 20.0) and 13% were overweight (BMI > 25.0). These percentages are comparable to Australian data[10]. The mean prestige ranking was 3.9 (SD 1.1) using Daniel's scale, corresponding to semi-professional and middle-management groups.

The FFQ was satisfactorily completed by 167 subjects after excluding under- and over-reporters. The median daily nutrient intakes were; energy 7700 kJ, 16.1% energy from (% E) protein, 52.2% E carbohydrate, 30.2% E fat, 1.9% E alcohol, 24.4 g fibre, 11.7 mg iron, 1.93 iron from meat, fish and poultry (MFP-iron), 1.07 mg haem iron, 830 mg calcium, 156 mg vitamin C. Nearly 25% of subjects reported never drinking alcohol. The median daily alcohol intake was 4.8g per consumer, with 4% of students consuming in excess of 20g alcohol daily.

A large proportion of subjects classified themselves as vegetarian (13%) or semi-vegetarian (17%), and consumed minimal amounts of red meat. The use of vitamin and mineral supplements was common, with 41% of subjects taking supplements on a regular basis. Thirty-five percent of subjects were currently using the OCP and none reported the use of an IUD. Most subjects had regular cycles (73%) and menstruated 4-5 days per cycle (60%). Using menstrual scores, the following percentages were obtained; 17% for low score, 48% for medium and 35% for high score. Thirteen percent of subjects had donated blood at least once in the past six months. The majority of students (66%) reported no or low levels of exercise and only 10% reported high levels of exercise, based on frequency, duration and intensity of activities undertaken.

Iron status

The mean values, 95% CI of the mean and percentiles for the iron status parameters measured are presented in Table 1. Various methods for estimating the prevalence of iron deficiency (ID) and iron deficiency anaemia (IDA) are compared in Table 2. Using multiple criteria, iron deficiency was present in 7.2% of the student sample and iron deficiency anaemia in 4.5%. A large percentage of students were anaemic (10.2%) although there was no other apparent cause of the anaemia in subjects who were not iron deficient. The prevalence of low iron stores (SF<20 μg/L) was 19.8%.

Table 1. Haematological and biochemical indicators of iron status in female students in Perth, aged 15-30 (n=265).

Parameter (reference range)	**Mean**	**95% CI**	**Percentile 2.5**	**10**	**50**	**90**	**97.5**
Hb (12.0-16.0 g/dL)	13.2	13.0-13.3	11.1	11.9	13.2	14.1	14.7
MCV (80-100 fL)	86	85.3-86.3	75	81	86	90	93
MCHC (31-36%)	34.4	34.3-34.5	33	33	34	35	36
SI (7-24 μmol/L)	16	15.1-16.7	4	8	16	25	31
Tn (2.0-4.0 g/L)	3.2	3.14-3.26	2.4	2.6	3.1	3.9	4.1
TS (16-40%)	26	24.2-27.1	6	11	24	42	51
SF * (20-200 μg/L)	28	25.1-30.4	4	12	29	68	101

* geometric mean for SF (serum ferritin)

Table 2. Prevalence of iron deficiency in 15-30 year old female students in Perth measured by various criteria (n=265).

Criteria	**%**	**(n)**
Iron deficiency Anaemia		
Single criterion (Hb<12.0)	10.2	(27)
Multiple criteria (Hb<12.0, SF≤12, TS<16)	4.5	(12)
Iron deficiency		
Single criterion		
SF≤12	12.5	(33)
SF<16 *	19.8	(51)
TS<16	19.8	(52)
Multiple criteria (SF≤12, TS<16)	7.2	(19)

* criterion according to Hallberg *et al*[17]

Multivariate analysis

Multivariate analysis was undertaken to examine the factors independently associated with iron status in this population. The predictor variables entered in the logistic regression model were: age, BMI, social status, recent blood donation, menstrual score, OCP use, vitamin/mineral supplement use, alcohol intake, exercise levels, energy intake (kJ), protein (% E), total iron density, haem iron density, calcium density and vitamin C density. The outcome variable was low iron stores (SF<20 μg/L). Table 3 presents the results of the most parsimonious logistic regression model with significant predictors of low iron stores being social status (high), haem iron density (low), calcium density (high), recent blood donation (yes), BMI (low) and menstrual score (high).

Table 3. Factors associated with low iron stores in students aged 15-30 year old

Variable	Coefficient	Std Error	OR	95% C.I.
Social status (per unit change)	-0.59	0.21	0.56	0.37-0.85
Calcium density (per 100mg/1 MJ change)	1.13	0.53	3.10	1.10-8.75
Haem iron density (per 0.1 mg/1 MJ change)	-0.45	0.23	0.64	0.41-1.00
Donation (compared to no donation)	19.06	8.20	-	-
Donation x BMI	-0.79	0.37	-	-
Menstrual score (compared to low/medium)				
high	1.0177	0.4588	2.77	1.13-6.80

Logistic regression analysis: deviance=150.8, df=160, n=167

A higher social status was associated with greater chances of low iron stores. Haem iron density was protective of iron stores. A diet containing 0.1 mg haem iron/MJ (the equivalent of 0.8 mg of haem iron in a 8000 kJ 'Western diet') decreases the odds of low stores by 35% compared to a vegetarian diet which contains no haem iron. A diet containing 1.6 mg of haem iron (approximately 100g lean beef) and 8000 kJ, would reduce the odds of low iron stores by 60%. Dietary calcium density is a positive predictor of iron deficiency in this model, with the odds of low iron stores being increased three-fold, with an increase in calcium density of 100 mg calcium/MJ. In practical terms, this is the equivalent of a change in calcium intake from 400 mg to 1200 mg, assuming a constant energy intake of 8000 kJ per day.

An interactive effect was observed between recent blood donation and BMI. A BMI greater than 24 was found to be protective against low iron stores for blood donors only, while a BMI below 24 increased the risk of low iron stores in blood donors. BMI was not associated with iron stores in non-donors. A high menstrual score (menstruating for more than 65 days per year) was associated with an increase in the odds of low iron stores of over 2.5 times compared with women who menstruated fewer days per year.

Factors which were not found to be associated with iron deficiency in this study included age, vitamin and mineral supplement use, oral contraceptive use, alcohol intake, exercise levels, energy intake, protein intake, total iron intake and vitamin C intake.

Discussion

The results of this study report the iron status and the factors predictive of low iron stores in a group of 15-30 year old female students in Perth. Anthropometric data were comparable to Australian data of similar populations[10]. The social status of the sample is relatively high when compared to the general population due to the large number of university students in the sample[18].

The prevalence of iron deficiency (TS<16 and SF≤12) in this sample of female students was 7.2%, and iron deficiency anaemia (Hb<12, TS<16 and SF≤12) was present in 4.5% of students. These results are comparable to Australian studies of iron status in women (Table 4). The prevalence of iron deficiency is lower in the present study (7.2%) compared to 15 year old schoolgirls (9.2%)[2] but higher when compared to 20-69 year old women (4%)[3].

Factors associated with low iron stores

Social status, as assessed by parental occupational prestige, was found to be inversely related to iron status, after controlling for other known risk factors. This is in contrast to the study by Leggett *et al* [8], who found higher than average iron stores in populations of high socioeconomic status. Social status is difficult to measure in university students, as university life is often a transient stage with many students leaving home for the first time, and being required to organise their own meals and become responsible for their finances. Parental occupational prestige may thus not be the ideal measure of the social status of the student, but was chosen due to the lack of alternative measures. A possible reason for an adverse association between social status and serum ferritin concentration may be a greater pre-occupation with body weight and image in young women from high prestige family backgrounds and/or who are high achievers[19].

The number of studies showing significant associations between diet and iron status is relatively small. This is probably due to the difficulties of evaluating dietary intake over an appropriate period of time as iron status is the balance between iron absorption and loss, usually over several months. Methods to assess intake over short periods of time, for example 24-hour recall, do not take into account the high day-to-day variability of food consumption. The FFQ, which evaluates dietary intake over a longer period of time, may be more appropriate for investigating the relationships between diet and iron status. Indeed, in the present study, two nutrient variables (haem iron density and calcium density) were found to significantly affect iron status. No relationship was found with vitamin C intake, a known enhancer of iron absorption.

Low haem iron densities were found to increase the chances of becoming iron deficient, after controlling for other co-variables (social status, calcium intake, BMI, blood

Table 4. Iron status of Australian women (data on 15-30 year old where available).

Reference	Subjects	n	Criteria used	Prevalence (%)	Mean levels
NHF, 1989[3]	20-69 y women Australia	4267	SF<10	8	
			TS<10	9	
			ID	4	
Leggett *et al*, 1990[8]	17-65 y female employees Brisbane	920	SF≤10 and TS<20	5.5	
			SF≤10	8.9	
English and Bennett, 1990[2]	15 y schoolgirls Australia	142	SF<12 and TS<16	9.2	SF=31.7
			SF<12	20	SI=16.9
			TS<16%	21	TS=22.3
This study	15-30 y students Perth	265	SF≤12 and TS<16	7.2	SF=28 *
			SF≤12	12.5	TS=26
			SF≤10	8.7%	Hb=13.2

* geometric mean

donation and menstrual score). Preziosi *et al*[20] found a similar relationship between haem iron intake and serum ferritin concentration in a French population. These results are also in agreement with current literature which suggests that low intakes of meat and fish are a risk factor for iron deficiency[4,8,21,22].

No relationship was found between dietary iron intake and serum ferritin concentration. This suggests that the quality of iron intake (haem iron versus non-haem iron) is a more important determinant of iron status than the quantity of iron consumed. Most other studies have failed to find a significant association between non-haem iron intake and serum ferritin concentration[23-25].

A high calcium intake was associated with an increased likelihood of becoming iron deficient, after controlling for other factors. A calcium-rich diet (1200 mg), as recommended by the US Consensus Statement on Calcium Intake[26], increases the odds of low iron stores three times when compared to a calcium-poor isocaloric diet (400 mg). A relationship between calcium intake and iron stores has been observed previously[20], as well as an association between a high consumption of dairy products and iron deficiency[23,27]. Recent data show that calcium inhibits haem and non-haem iron absorption when consumed simultaneously[7].

Blood donation is a well known risk factor for iron deficiency[8,15,28]. Fogelholm *et al*[15] showed that women who had donated blood in the past 6 months were 2.5 times more likely to have low iron stores. In the present study, recent blood donation was found to be a significant predictor of low iron stores, but only in subjects with a BMI<24. For an individual of small body size, the donation of a unit of blood (240 mg iron) represents a larger proportion of total body iron which may lead more rapidly to smaller body iron stores. Further evidence of this has been provided by Monsen *et al*[29] who described the profile of a super-donor (frequent blood donor) as being of large body.

An increased number of menstruating days per year (>65 days) was associated with a 2.5 times increased likelihood of iron deficiency, compared to fewer menstruating days (<65 days). Evidence of an inverse association between serum ferritin concentration and the duration of menses has been provided by other investigators[15,23,30]. OCP use was not found to be significantly associated with iron deficiency in the present model. However, as the OCP reduces the duration of menstruation, its effect may have already been accounted for in the menstrual score.

The iron status of students was found to be comparable to the iron status of premenopausal women surveyed in recent Australian studies. However, a relatively large proportion of women (one in five) had low iron stores as defined by a serum ferritin <20 μg/L. The factors affecting iron stores were social status, haem iron intake, calcium intake, BMI, recent blood donation, and menstrual score. Haem iron intake decreased the likelihood of becoming iron deficient, whereas a high calcium intake, high social status, high menstrual score and a recent history of blood donation by subjects with BMI<24 increased the likelihood of becoming iron deficient. Of all these factors, increasing haem iron intake is the most appropriate and easily modifiable factor for public health intervention. In order to decrease the prevalence of iron deficiency in this population, haem iron consumption (meat, chicken fish) should be increased, but separately from the main calcium containing meals. Further research is necessary to determine whether iron stores are affected by separating high calcium meals from high iron meals.

Acknowledgment. This research was conducted at the School of Public Health, Curtin University of Technology, Perth, where the first author is undertaking doctoral research. This research was supported in part by a grant from Kellogg (Aust) Pty Ltd. The authors would like to thank the staff at the Health Service, Curtin University of Technology for their valuable assistance.

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Asia Pacific Journal of Clinical Nutrition (1997) Volume 6, Number 4: 291-295

影響15-30歲女學生鐵營養狀況的因素

摘要

在澳洲有關影響年青婦女鐵營養的因素和鐵營養狀況的數據是不多的. 本研究選15-30歲女學生爲對象, 用標準血液學和生化學試驗進行評估鐵營養狀況. 從人口統計結果數據及已知的缺鐵危險因素; 包括食物. 與已發表的澳洲數據比較, 鐵缺乏占7.2%, 而缺鐵性貧血占4.5%. 用對數回歸分析, 發現與鐵儲存低(血清鐵蛋白<20微克/升〕, 社會活動頻繁, 攝取血紅素鐵低, 攝取鈣高, 月經過多和最近有輸血史的BMI<24的婦女有關. 所有這些因素中, 增加血紅素鐵的攝取(進食肉類, 鷄和魚類〕是最適當的公共衛生干預.

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