Original Article

Iron deficiency and risk factors in pre-menopausal females living in Auckland, New Zealand

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Background and Objectives: Iron deficiency is prevalent in New Zealand, with low dietary haem intake and blood loss previously identified as risk factors. However, the influence of the hormone hepcidin on iron status has not been investigated. **Methods and Study Design:** Females (n=170) aged 18-45 residing in Auckland participated in a cross-sectional study. Iron status and inflammation were assessed with serum biomarkers including; serum ferritin, haemoglobin, soluble transferrin receptor, hepcidin, C-reactive protein and interleukin-6. Lifestyle factors were assessed using a series of validated questionnaires, including an iron food frequency questionnaire. Potential determinants of serum ferritin were identified using multiple linear regression analysis. **Results:** Iron insufficiency was confirmed in 55.8% of participants (Serum ferritin <30 µg·L-1). Hepcidin levels were higher in those who were iron sufficient (Serum ferritin ≥30 µg·L-1) (6.62 nM vs 1.17 nM, p<0.001). South Asian females had higher hepcidin (8.78 nM) levels, compared to New Zealand Europeans (6.28 nM) (p=0.018), a result likely due to South Asians presenting with higher interleukin-6 (1.66 vs 0.63 pg·mL-1, p<0.001). Hepcidin (β=0.082, p<0.001) and frequency of meat intake (β=0.058, p=0.001) were identified as significant predictors of serum ferritin in New Zealand Europeans, while hepcidin was the only identified predictor in South Asians (β=0.138, p<0.001) and those of other ethnicities (β=0.117, p<0.002). **Conclusions:** This is the first study in New Zealand to show that hepcidin levels strongly predict serum ferritin in premenopausal females. Additionally, frequency of meat intake appears to be an important determinant of iron status in New Zealand Europeans.

Key Words: iron insufficiency, hepcidin, inflammation, diet, ethnicity

INTRODUCTION

Iron deficiency (ID) is common in children, pregnant women and premenopausal females worldwide.[1] Previous research has suggested high rates of ID (serum ferritin (Sf) <12 µg·L-1 and zinc protoporphyrin >60 µmol·mol-1) within New Zealand (NZ), with a prevalence of 12.1% seen in 31-50 year old females.[2] Ethnic cohorts within NZ, such as Māori, Pacifica and Asians have been identified at a higher risk of developing ID compared to NZ Europeans.[2-4]

There are numerous factors known to contribute to ID.[5] The most recent (2008/09) NZ National Nutrition Survey indicated that 15.4% of females aged 31-50 years had an iron intake below the estimated average requirement of 8mg·day-1.[2] Additionally, vegetarianism has risen 27% since 2008/09, with one in ten New Zealanders now following a vegetarian style diet.[6,7] This dietary pattern is likely to contribute to the increased risk of ID due to non-haem iron (e.g. from dark leafy greens) being poorly absorbed as compared to haem iron, which is mainly found in red meat and animal products.[5,8] Blood loss has also been identified as a risk factor for poor iron status.[3,9-11] Within NZ premenopausal females, primary contributors to blood loss include blood donation, menstrual losses, and nose bleeds, with recent blood donation being the strongest risk factor for poor iron status.[3,9]

An area of emerging research in iron regulation and metabolism that is yet to be explored in non-athletic NZ females, is the role of the hormone hepcidin. Hepcidin is a peptide hormone that is known to inhibit the movement and utilisation of iron within the body by reducing iron export from enterocytes and recycling from macrophages.[12] Hepcidin reduces iron export by directly binding to iron export channels (ferroportin) on cell surfaces. This interaction of hepcidin and ferroportin results in the internalisation and lysis of the iron export channels and subsequently disrupts the flow of iron into the plasma.[12] In instances of elevated hepcidin activity, iron is sequestered in enterocytes, thereby lowering the amount of dietary iron moved through the gut and into the systemic circulation.[13-15]

Hepcidin levels in females have been shown to vary between countries and ethnicities.[16] For example, in healthy

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American and Italian females, the normal range for hepcidin was reported at 6.10-102.5 nM while Eastern European females had an average hepcidin concentration of 1.69 nM.[16,17] Furthermore, the expression of hepcidin within the ethnically diverse population of NZ females has not been investigated, so the extent to which hepcidin affects iron utilisation in females of different ethnicities is still unknown.

One of the factors that is associated with increased hepcidin concentrations is inflammation.[18] In particular, the inflammatory marker interleukin-6 (IL-6) is known to directly bind to the hepcidin gene (HAMP) to upregulate hepcidin transcription.[15] Individuals with high levels of inflammation may be at increased risk of having a poor iron status,[19] due to elevations in hepcidin inhibiting effective iron movement in the body.[20] Another prominent health condition in NZ associated with an inflammatory state is obesity.[21]

New Zealand has one of the highest obesity rates in the world, with one in three adults classified as obese.[22] A high BMI (≥30 $kg \cdot m^{-2}$) has been associated with elevated hepcidin levels, and is thought to be linked to increased expression of IL-6, therefore suggesting obesity is a risk factor for ID.[21,23] However, the relationship between body composition and iron status has not been investigated in NZ females, so the extent to which obesity contributes to NZ's ID rates is unknown.

The high incident rates of ID within NZ premenopausal females of various ethnicities highlights the need for further investigation on additional risk factors that contribute to ID as well as the influence of hepcidin expression on iron metabolism. Therefore, this study investigated the current iron and hepcidin status of premenopausal females in the Auckland region. The aim of this study was to determine the relationship between iron status, hepcidin and potential determinants (inflammation, dietary intake and body composition) that would increase the risk of ID in females.

METHODS

This study was a cross-sectional study conducted in premenopausal females residing in Auckland, NZ. Data collection commenced in July 2018 and concluded in July 2019.

Participants and recruitment

Participants were recruited through Massey University (Albany campus, NZ), social media and community groups. Initial recruitment targeted South Asian community groups as they have previously been shown to have high rates of ID.[24] Prior to each testing session participants were screened to ensure they met the inclusion criteria. Premenopausal females 18-45 years of age and currently residing in Auckland NZ were eligible to take part. Participants were excluded from the study if they had consumed iron supplements (>20 mg elemental iron) three or more times per week in the last three months, or if they had given blood or received a blood transfusion in the past six months. Females who were currently breastfeeding or pregnant (including pregnancy in the last year) and those who had chronic diseases that are known to affect iron status such as inflammatory bowel disease, chronic kidney disease or coeliac disease were also excluded.

The sample size was calculated from the following formula: $n = [Z2\ p(1-p)]/d2]$. Based on an estimated prevalence of ID of 12.1% in the population of interest,[2] a 5% precision and 95% level of confidence giving a corresponding z-score of 1.69, a sample of 162 participants was determined to be adequate.

Ethics approval was obtained from Massey University Human Ethics Committee: Southern A (18/12) and all participants provided written informed consent prior to participating.[25,26]

Data collection was completed at Massey University's Auckland campus or researcher approved community centres. During a single data collection session, participants underwent a body composition analysis, venous blood collection, and completed a series of online questionnaires.

Body composition measurements

Height was measured to the nearest centimetre using a stadiometer. Body composition was determined using bioelectrical impedance analysis (InBody 230). The data of interest included total body weight and body fat percentage. Body mass index was calculated using the participant's weight (in kilograms) divided by height (in meters) squared.

Blood sample analysis

A single finger prick blood sample was collected to measure haemoglobin using the HemoCue® Hb 201+ System. A venous blood sample was collected by a trained phlebotomist from the antecubital vein with the participant in a seated and rested position. The blood samples were collected using a sterile 21–gauge flashback needle into two 5-mL SST gel separator tubes and one 3-ml EDTA tube. The SST tubes were allowed to clot for 30-60 minutes at room temperature before being centrifuged at 10°C, 1000rcf for 10 minutes. The serum supernatant was removed and divided into 1-ml aliquots and stored at -80°C until analysis. Once all blood samples were collected, serum was sent to Auckland LabPlus and Canterbury Health laboratory for markers of iron status analysis and inflammation (C-Reactive Protein (CRP), Sf and soluble transferrin receptor (STfR). Serum interleukin-6 (RD Systems Human IL-6 Immunoassay high sensitivity ELISA, D6050) and hepcidin (RD System Human Hepcidin Immunoassay ELISA, HDP250) were analysed via commercially available ELISA kits at Massey University.[27,28]

Health, demographic and food frequency questionnaires

Questionnaires were conducted online using the software Qualtrics. The questionnaire was an amalgamation of previously validated questionnaires that collected self-reported information on the participant's demographics (e.g. age, ethnicity) and medical history (e.g. medication, contraceptive choice, previous chronic disease and/or ID). Dietary intake (at the food group level) and menstrual blood loss were determined using previously validated iron food frequency and menstrual blood loss questionnaires.[29,30] Within the food frequency questionnaire, meat

intake included all poultry, red meat (e.g. beef, lamb, venison), pork, game meats, processed meats, and seafood.

Data handling and statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics 25 for Windows (IBM Corporation, Armonk, NY, USA). Data was tested for normality using Kolmogorov-Smirnov and Sharpiro-Wilk tests, with $p>0.05$ for either test treated as normally distributed. Data that did not meet the criteria for normality was log transformed and retested. Log transformed data that met the criteria for normal distribution is presented as it's geometric mean and 95% confidence interval. Normally distributed data is presented as mean ± standard deviation, while non-normal data is presented as median (25% and 75% percentiles). Categorical data is reported as number of participants and percentages for each group.

Comparison between participants for parametric continuous data was conducted through independent t-tests and one-way ANOVA. Non-parametric data was compared using Mann-Whitney U and Kruskal Wallis tests. Pearson's Chi-squared test was used to compare categorical variables. For chi-squared test, the expected count for each cell was ≥5, and all groups were independent. For all tests, a *p*-value of ≤0.05 was considered as statistically significant. For significant differences between groups determined using one-way ANOVA; Tukey's HSD post hoc test was used to identify where the difference occurred. For significant differences between groups identified by Kruskal Wallis' test; the Mann-Whitney U test was used to identify where the difference occurred.

Multiple linear regression analysis was used to identify the relationship between Sf and potential risk factors for ID. Frequency of food group consumption (alcohol, tea & coffee, dairy, meat, iron fortified foods, cereals, fruit & vegetable) intake per week were entered as continuous variables. Age, body fat percentage and length of period were also entered as continuous variables. Having children, contraceptive method, previous ID and previous blood donation were entered as categorical variables.

As Sf was the dependent variable, and was not initially normally distributed, a natural log transformation was used to meet the assumption for multiple linear regression analysis. There was no multicollinearity between independent variables as tested by variance inflation factor, with <10, and a tolerance >0.2 indicating no multicollinearity. Additionally, Pearson's correlation co-efficient was used to check for correlations between independent variables, with values of ≤0.80 treated as lack of correlation. All residuals were independent as tested by the Durbin-Watson test, with a test statistic of 1-3 indicating no correlation between residuals. The residual scatterplot was reviewed for homoscedasticity, and confirmed by a lack of pattern in the distribution in the residual scatterplot.

RESULTS

Participant characteristics

Of the 170 females recruited, five were excluded as no blood samples were able to be collected, therefore, 165 females were included in the final analysis. Of those included in the analysis, 73 (44.2%) had sufficient iron stores (Sf ≥30 μg·L-1 and Hb ≥120 g·L-1) and 92 (55.8%) had insufficient iron stores (Sf <30 μg·L-1; Hb <120 g·L-1 or ≥120 g·L-1), of which 14 (8.5%) had iron deficiency anaemia (Sf ≤12 μg·L-1; Hb <120 g·L-1). Previous research within NZ has used a cut-off of Sf of <20 μg·L-1 to define iron insufficiency. For comparison, using a cut off for Sf <20 μg·L-1, 93 (56.4%) participants were iron sufficient and 71 (43.0%) were iron insufficient. Forty-nine (29.7%) participants had a CRP ≥5 mg·L-1, and a correction factor was used to adjust their Sf values.[31]

Table 1 summarises the characteristics of the participants. The largest ethnic group was South Asians (n=70; 42.4%), followed by NZ Europeans (n=62; 37.6%). Those of other ethnicities were the smallest group (n=33; 20.0%) and included other Asian, Maori, Pacifica and Middle Eastern ethnicities. The median age of participants was 27 (22, 36) and 26 (21, 32) years for iron sufficient and insufficient respectively. In terms of frequency per week, consumption of different food groups did not differ significantly between those that were iron sufficient and those that were insufficient, with the exception of meat intake. Frequency of meat intake was significantly higher (p=0.006) in those with sufficient iron stores (9.21 times per week) than those with insufficient iron stores (6.29 times per week). Other demographic and health characteristics did not differ significantly between participants with sufficient and insufficient iron scores.

Table 2 shows that participants with insufficient iron stores had significantly lower Hb (128 g·L-1 vs 133g·L-1) (p=0.011), Sf (15.8 μg·L-1 vs 60.8 μg·L-1) (p<0.001), and hepcidin (1.71 nM vs 6.62 nM) (p<0.001) than those with sufficient iron stores. Participants with insufficient iron stores had a higher STfR (3.34 mg·L-1 vs 2.55 mg·L-1) (p<0.001) and sTfR/log ferritin ratio (2.72 vs 1.50) (p<0.001). There was no difference in inflammatory markers, interleukin-6 and C-reactive protein, between participants with insufficient and sufficient iron stores.

Table 3 summarises the biomarkers, body composition and meat intake between ethnic cohorts. Haemoglobin differed significantly between ethnicities for those participants who had sufficient (p=0.025) and insufficient iron stores (p<0.001). South Asians with sufficient iron levels had significantly lower Hb levels (129 g·L-1) than NZ Europeans with sufficient iron levels (137 g·L-1) (p=0.019). Similarly, South Asians with insufficient iron levels had significantly lower Hb (122 g·L-1) than NZ Europeans (136 g·L-1) (p<0.001) and those of other ethnicities (132 g·L-1) (p=0.014) with insufficient iron levels.

Soluble transferrin receptor differed significantly between ethnicities, however, only in individuals classified as iron insufficient (p=0.017). South Asians with insufficient iron levels had a significantly higher STfR (3.63 mg·L-1) than those of other ethnicities with insufficient iron levels (2.77mg·L-1) (p=0.045).

Hepcidin only differed significantly between ethnicities for those participants who were iron sufficient (p=0.026). South Asians with sufficient iron levels had a higher serum hepcidin (8.78 nM) than both NZ Europeans (6.28 nM) (p=0.025) and other ethnicities who were iron sufficient (4.89 nM) (p=0.018).

Table 1. Demographics and dietary intake of iron sufficient and insufficient participants

Characteristic	Iron sufficient† n=73 (44.2%)	Iron insufficient‡ n=92 (55.8%)	*p*-value
Age (years) §	27.0 (22.0, 36.0)	26.0 (21.0, 32.0)	0.273
BMI (kg·m^2)¶	24.4 (23.3, 25.6)	24.2 (23.3, 25.2)	0.753
Body fat percentage††	32.3 (9.79)	32.9 (9.78)	0.720
Period length (days) §	5.00 (4.00, 6.00)	5.00 (4.00, 6.00)	0.511
Ethnicity‡‡			
South Asian	25 (34.2)	45 (48.9)	
NZ European	30 (41.1)	32 (34.8)	0.141
Other	18 (24.7)	15 (16.3)	
Having children‡‡	23 (31.9)	25 (27.5)	0.534
Contraceptive choice‡‡			
Oral	14 (19.4)	14 (15.4)	
Other	13 (18.1)	12 (13.2)	0.477
None	45 (62.5)	65 (71.4)	
Previous Iron Deficiency‡‡	35 (49.3)	39 (43.8)	0.490
Previous Blood Donation (ever)‡‡	5 (7.0)	9 (10.0)	0.508
Self-reported dietary patterns‡‡			
Normal	56 (77.8)	57 (62.6)	
Vegetarian/Vegan	8 (11.1)	16 (17.6)	0.113
Other	8 (11.1)	18 (19.8)	
Alcohol (p/w) §	0.71 (0.00, 2.17)	0.42 (0.0, 1.67)	0.458
Dairy (p/w) §	18.4 (10.1, 28.4)	19.4 (9.3, 28.0)	0.860
Tea and coffee (p/w) §	9.25 (1.75, 18.29)	6.00 (1.08, 15,0)	0.152
Meat (p/w) §	9.21 (5.50, 13.96)	6.29 (2.25, 9.83)	0.006
Iron fortified cereals (p/w) §	1.67 (0.33, 4.33)	1.33 (0.50, 5.67)	0.739
Fruits and vegetables (p/w) §	52.4 (33.7, 64.9)	53.2 (37.3, 73.8)	0.504
Cereals and Grains (p/w) §	8.17 (3.58, 13.0)	8.54 (3.75, 13,.8)	0.882

p/w: number of times consumed per week.
†Serum ferritin ≥30 μg·L^{-1} and Hb ≥120 g·L^{-1}
‡Serum ferritin <30 μg·L^{-1} (Hb <120 g·L^{-1} or ≥120 g·L^{-1})
§Median and 25th, 75th centiles
¶Geometric mean and 95% confidence interval
††Mean±standard deviation
‡‡Number and % of participants

Table 2. Biochemical markers of iron status and metabolism in iron sufficient and insufficient participants

Biomarker	Iron sufficient† n=73 (44.2%)	Iron insufficient‡ n=92 (55.8%)	*p*-value
Haemoglobin (g·L^{-1}) §	133 (12.0)	128 (13.0)	0.011
Serum ferritin (μg·L^{-1}) ¶	60.8 (38.00, 71.00)	15.8 (9.00, 23.0)	<0.001
Soluble transferrin receptor (mg·L^{-1}) ††	2.55 (2.14, 2.69)	3.34 (3.14, 3.55)	<0.001
sTfR/log ferritin ratio¶	1.50 (1.30, 1.80)	2.72 (2.10, 3.80)	<0.001
C-Reactive protein¶	0.00 (0.00, 0.00)	0.00 (0.00, 3.00)	0.075
Interlukein-6 (pg·mL^{-1}) ¶	0.95 (0.54, 1.49)	1.15 (0.62, 1.87)	0.125
Hepcidin (nM) ¶	6.62 (4.21, 11.02)	1.71 (0.59, 3.47)	<0.001

†Serum ferritin ≥30 μg·L^{-1} and Hb ≥120 g·L^{-1}
‡Serum ferritin <30 μg·L^{-1} (Hb <120 g·L-1 or ≥120 g·L^{-1})
§Mean±standard deviation
¶Median and 25th, 75th centiles
††Geometric mean and 95% confidence interval

Serum ferritin, sTfR/log ferritin ratio and C-reactive protein did not differ significantly between ethnicities, regardless of iron status.

Interlukein-6 differed significantly (p<0.001), with South Asians presenting with higher serum IL-6 (1.66 pg·ml^{-1}) than NZ Europeans (0.63 pg·ml-1) (p<0.001) as well other ethnicities (0.80 pg·ml-1) (p<0.001).

There were significant differences in both BMI (p=0.001) and body fat percentage (p≤0.001) between ethnic groups. South Asians (26.3 kg·m^{-2}) had a significantly higher BMI than NZ Europeans (23.2 kg·m^{-2}) (p=0.001). South Asians (39.1%) also had a significantly higher body fat percentage than NZ Europeans (27.4%) (p≤0.001) and those of other ethnicities (30.7%) (p≤0.001).

Meat intake differed between ethnicities (p<0.001). Frequency of meat intake was significantly lower (p<0.001) in South Asians (3.5 times per week) when compared to other ethnicities (11.25 times per week) and NZ Europeans (8.33 times per week) (p=0.001). This difference in meat intake may be explained by significant differences in eating pattern between ethnicities (p<0.001). South Asians had 5.28 higher odds of being vegetarian than NZ Europeans, and 4.86 times higher

Table 3. Comparison of iron status and factors that increase the risk of iron deficiency between different ethnicities

Characteristic	New Zealand European n=62 (37.6%)		South Asian n=70 (42.4%)		Other n=33 (20.0%)		*p*-value	
	Sufficient†	Insufficient‡	Sufficient†	Insufficient‡	Sufficient†	Insufficient‡	Sufficient†	Insufficient‡
Iron sufficient/insufficient§	30 (48.4)	32 (51.6)	25 (35.7)	45 (64.3)	18 (54.5)	15 (45.5)	0.141	
Haemoglobin ($g \cdot L^{-1}$)¶	137 (11.0)	136 (10.0)	129 (12.0)	122 (12.0)	134 (9.00)	132 (10.0)	0.025	<0.001
Serum ferritin ($\mu g \cdot L^{-1}$)††	44.8 (39.0, 58.0)	16.1 (10.0, 22.1)	48.0 (32.6, 79.0)	14.0 (7.21, 22.5)	62.0 (43.7, 103)	15.0 (9.00, 24.0)	0.259	0.749
Soluble transferrin receptor ($mg \cdot L^{-1}$)ϕ	2.64 (2.41, 2.89)	3.13 (2.89, 3.39)	2.48 (2.27, 2.69)	3.63 (3.29, 4.01)	2.51 (2.18, 2.89)	2.97 (2.55, 3.42)	0.573	0.017
sTfR/log ferritin ratio††	1.60 (1.30, 1.90)	2.55 (2.05, 3.40)	1.43 (1.30, 1.70)	3.04 (2.20, 4.77)	1.40 (1.20, 1.70)	2.60 (1.80, 3.70)	0.278	0.103
Hepcidin (nM)††	6.28 (3.78, 9.88)	1.08 (0.54, 3.56)	8.87 (6.22, 15.1)	1.73 (0.48, 3.50)	4.89 (3.74, 8.47)	1.77 (1.16, 2.23)	0.026	0.944
C-reactive protein ($mg \cdot L^{-1}$)††	0.00 (0.00, 3.00)		0.00 (0.00, 4.00)		0.00 (0.00, 0.00)		0.099	
Interlukein-6 ($pg \cdot mL^{-1}$)††	0.63 (0.45, 1.01)		1.66 (1.26, 2.44)		0.80 (0.57, 1.12)		<0.001	
BMI ($kg \cdot m^2$)‡‡	22.9 (22.1, 23.7)		25.7 (24.7, 27.0)		23.8 (22.0, 25.8)		0.001	
Body fat percentage¶	27.4 (7.58)		39.1 (11.1)		30.7 (9.30)		<0.001	
Self-reported dietary patterns ‡‡								
Normal	54 (83.1)		36 (49.3)		31 (25.6)			
Vegetarian/Vegan	5 (7.7)		17 (23.3)		3 (8.6)		<0.001	
Other	6 (9.2)		20 (27.4)		1 (2.9)			
Meat (p/w)††	8.33 (5.75, 11.3)		3.50 (0.0, 8.67)		11.25 (7.08, 16.4)		< 0.001	

BMI: body mass index; p/w: Number of times consumed per week.
†Serum ferritin ≥30 $\mu g \cdot L^{-1}$ and Hb ≥120 $g \cdot L^{-1}$.
‡Serum ferritin <30 $\mu g \cdot L^{-1}$ (Hb <120 g·L-1 or ≥120 $g \cdot L^{-1}$).
§Number and % of participants.
¶Mean ± standard deviation.
††Median and 25th, 75th centiles.
‡‡Geometric mean and 95% confidence interval.

odds than other ethnicities. Other ethnicities had 1.08 times higher odds of being vegetarian than NZ Europeans. Frequency of intake of other food groups did not differ significantly between ethnicities.

Overall predictors of serum ferritin

There was multicollinearity between ethnicity and meat intake, therefore, multiple linear regression analysis was used to identify significant predictors of Sf within each ethnic group. Predictor variables were selected by first testing each individual variable, and those with a $p<0.200$ were then entered into the final model using the enter method. The following variables did not meet the screening criterion, so were not entered into the multiple regression analysis: body fat percentage, period length, having children, contraceptive method, previous ID, blood donation, alcohol intake, dairy intake, tea & coffee intake, iron-fortified food intake, fruits & vegetable intake, grains & cereal intake and serum IL-6. Predictors that were entered into the model included frequency of meat intake as well as serum hepcidin levels.

The model suggested that hepcidin and frequency of meat intake accounted for 42.0% of the variation in Sf in NZ Europeans. The model indicated that hepcidin status was a stronger predictor of Sf levels than frequency of meat intake. This model demonstrated when frequency of meat intake increased by one time per week, the log of Sf increased by 0.058 (1.06 $\mu g \cdot L^{-1}$).

Meat intake was not a significant predictor of Sf in either South Asians, or those of other ethnicities. The model indicated that hepcidin accounted for 56.2% of the variation in Sf for South Asian participants, and for those of other ethnicities, hepcidin accounted for 27.0% of the variation in Sf. Hepcidin was positively associated with Sf in all ethnic groups.

DISCUSSION

To the best of our knowledge, we are the first to analyse iron and hepcidin status in non-athletic NZ females, in a cohort with a high representation of South Asian participants, an ethnic group that have been shown to have a high incidence of ID (Figure 1).[24]

Prevalence of iron deficiency in Auckland premenopausal females

The overall prevalence of ID in our cohort was high (55.8%) compared to previous research in NZ premenopausal females.[3,9] A previous study undertaken in 18-44 year old females in Auckland found 18.7% of participants had suboptimal iron stores (Sf <20 $\mu g \cdot L^{-1}$).[3] Similarly, a study in 18-40 year old premenopausal females in Dunedin, indicated that 23% had mild ID (Sf <20 $\mu g \cdot L^{-1}$).[9] The NZ National Nutrition Survey indicated the highest prevalence of ID (Sf <12 $\mu g \cdot L^{-1}$) was found in women aged 31-50 years at 12.1%.[2] Although we used a higher cut off for ID (Sf <30 $\mu g \cdot L^{-1}$) than previous studies, when we used a similar cut off to previous research in NZ, of Sf <20 $\mu g \cdot L^{-1}$, the rates of ID (43.0%) of the current study were still comparatively high.

One possible explanation for the high rates of ID in our study was the high rates of vegetarianism amongst participants. Our study had 30.3% of all participants excluding certain types of meat (e.g. being pescatarian), and 14.5% of all participants were either vegetarian or vegan. A similar study in Dunedin premenopausal females also found that 22% of participants avoided red meat.[9] Our results were higher than rates from the National Nutrition Survey, which showed 7.7% of 19-30 year old and 6.4% of 31-50 year old females had not consumed red meat within the past four months.[2] The increase in vegetarianism appears to be a generational trend, with the most likely age groups to be vegetarian in NZ being those aged 25-34 (13.8%) closely followed by 14-24 year olds (13.3%). The mean age groups of iron sufficient and insufficient of this study were 27 and 26 years respectively, therefore it is likely that our cohort are representative of this generational change to a vegetarian based dietary pattern within New Zealand .[6]

Another potential explanation for the high rates of ID was our high representation of South Asian participants (42.4%). Our study had 37.5% NZ Europeans as compared to 95% and 70% in previous research done in NZ females.[3,9] The most recent NZ National Nutrition Survey indicated that South Asians were more likely than any other ethnic group to have low iron stores (Sf ≥12 $\mu g \cdot L^{-1}$).[24] Furthermore in Auckland, Asian premenopausal females, (the majority of whom were either of Chinese (41.8%) or Indian (29.1%) ethnicity), had five times

Table 4. Multiple linear regression analysis to identify predictors of iron deficiency stratified by ethnicity

	β	95% CI for β	Standardised β	*p*-value
New Zealand European				
Hepcidin (nM)	0.082	0.044, 0.121	0.454	<0.001
Meat Intake	0.058	0.026, 0.091	0.382	0.001
		F (2, 56)=19.5 $p \leq 0.001$ R^2=0.420		R^2 (adj)=0.398
South Asian				
Hepcidin (nM)	0.138	0.107, 0.168	0.750	<0.001
		F (1, 66)=83.5 $p \leq 0.001$ R^2=0.562		R^2 (adj)=0.555
Other ethnicities				
Hepcidin (nM)	0.117	0.045, 0.189	0.519	0.002
		F (1, 31)=11.1 p=0.002 R^2=0.270		R^2 (adj)=0.245

Frequency of food group consumption (alcohol, tea & coffee, dairy, meat, iron fortified foods, cereals, fruit & vegetable) intake per week were entered as continuous variables. Age, body fat percentage and length of period were also entered as continuous variables. Having children, contraceptive method, previous ID and previous blood donation were entered as categorical variables.

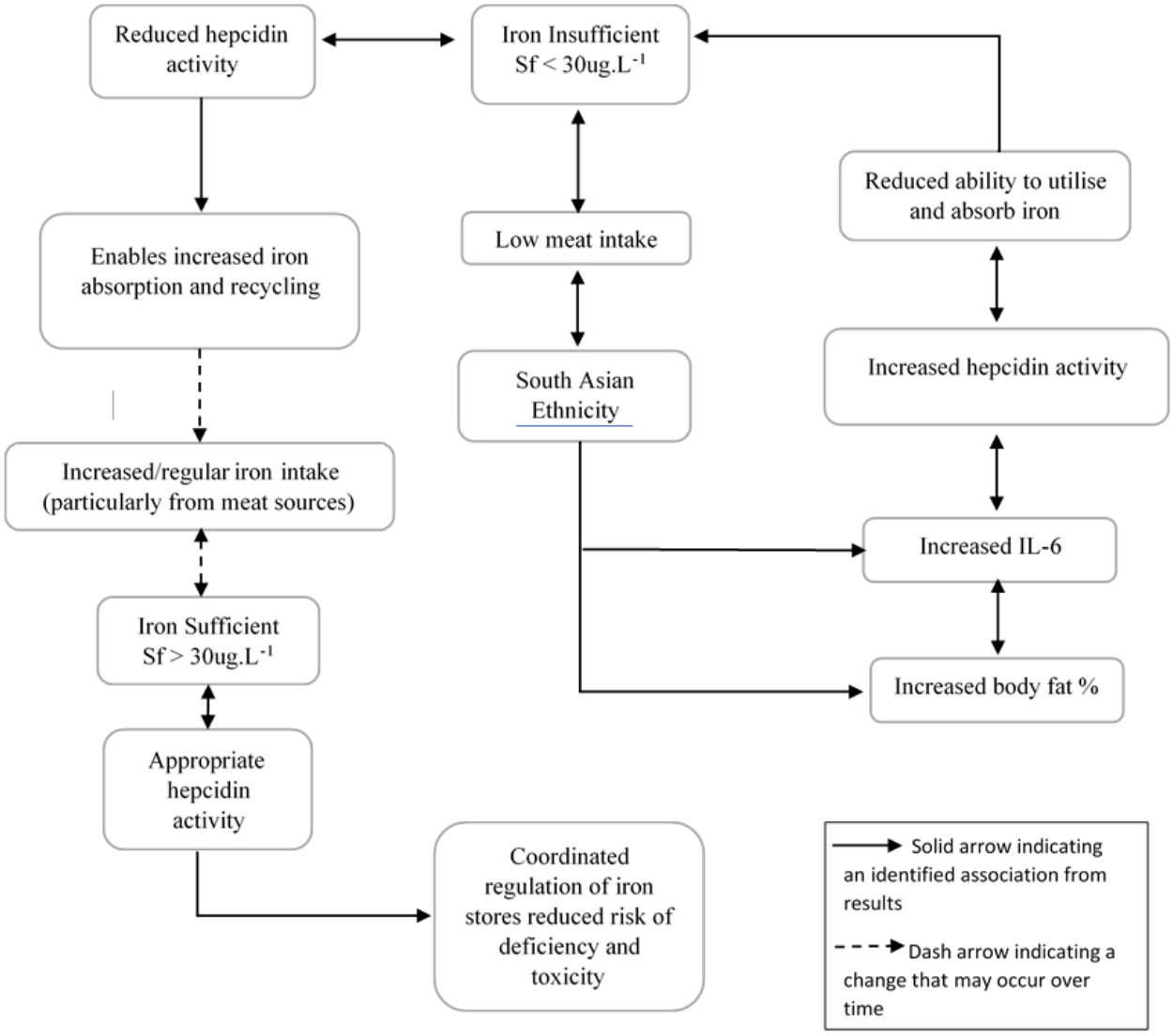

Figure 1. Conceptual diagram of associations between body composition, dietary intake and the influence of the hormone hepcidin iron status in premenopausal females. Females of South Asian ethnicity were identified as a high-risk cohort for the development of iron insufficiency due to a lower intake of meat and elevated hepcidin levels associated with increased body fat % and elevated IL-6. However, iron stores were the strongest determinant of hepcidin activity, with iron insufficiency reducing the hepcidin response, thus limiting the risk of iron deficiency. A higher intake of meat intake may also aid in the restoration of iron stores.

higher odds of having ID than NZ Europeans, a result that would appear to be reflected in our study.[3]

Ethnic differences in iron status

For the analysis of iron status between and within ethnic groups, results were separated into iron sufficient/insufficient. This was deemed appropriate as hepcidin activity is reduced and frequently observed to be 'non-respondent' iron insufficient individuals, this is owing to the homeostatic control that iron stores (Sf) has on hepcidin activity.[32] Due to the high rates of iron insufficiency within the current cohort an overall average of Sf and hepcidin within each ethnic group would confound meaningful results in determining the factors that affect iron status in females in New Zealand.

Although not statistically significant, our study showed that South Asians had 1.68 higher odds of having ID than NZ Europeans and 2.16 higher odds of having ID than those of other ethnicities. This could potentially be explained by ethnic differences in body composition and inflammation leading to variations in hepcidin levels.

South Asians had a significantly higher BMI (26.3 $kg \cdot m^{-2}$) than NZ Europeans (23.2 $kg \cdot m^{-2}$). Although the difference in BMI was relatively small (3.1 $kg \cdot m^{-2}$), there was a large difference in body fat percentage between the two ethnicities (39.1% vs 27.4% for South Asians and NZ Europeans respectively). Similarly, South Asians also had a significantly higher body fat percentage than those of other ethnicities (30.7%).

Differences in body composition between ethnicities has also been demonstrated in previous research.[33–35] For the same BMI, age and gender, Asians have been shown to have a higher body fat percentage than Caucasians, with South Asians having the highest body fat percentage among Asian cohorts.[35] It is estimated that Indian women in NZ have around a 8% higher body fat percentage than Caucasians.[36] Some suggested explanations for these discrepancies in body composition include a combination of environmental factors (such as diet and exercise), genetics and even intrauterine development; as a 'thin-fat' phenotype has been found to be present from childhood in Asian Indian children.[33,35]

The higher body fat percentage found in both iron sufficient and insufficient South Asians could also be an underlying explanation for why South Asians had significantly higher serum IL-6 levels (1.66 $pg \cdot mL^{-1}$) than both NZ Europeans (0.63 $pg \cdot mL^{-1}$) and other ethnicities (0.80 $pg \cdot mL^{-1}$) in our study.

Previous studies in South Asian females have also shown significantly higher IL-6 (1.94 $mg \cdot L^{-1}$) than Europeans (1.51 $mg \cdot L^{-1}$).[37] It is estimated that 15-35% of IL-6 found in the blood originates from adipose tissue, and higher levels of IL-6 have previously been observed in individuals with obesity.[38] This research indicates that increased body fat may be a contributor to consistently elevated hepcidin, and therefore an increased risk of ID in this population.

A positive correlation between BMI and serum hepcidin has been demonstrated in Dutch females.[39] Conversely, a decrease in serum hepcidin concentration has been observed in bariatric surgery patients in response to weight loss.[40] Therefore, the higher IL-6 concentrations could explain why South Asians had a higher serum hepcidin (8.78 nM) than both NZ Europeans (6.28 nM) and those of other ethnicities (4.89 nM). The significantly higher hepcidin, IL-6 and body fat levels in South Asians that were iron sufficient would indicate that this cohort is at an increased risk of developing ID. Our results would suggest that South Asian females body composition may be favouring an inflammatory state and facilitating significantly higher hepcidin activity, that would be preventing optimal iron absorption in the gut and recycling of iron from macrophage activity and overtime would be contributing to the higher levels of ID seen in this cohort.

As previously stated, iron status has been identified as the strongest predictor of hepcidin expression.[32] Our results support this as individuals that presented with low Sf had a diminished hepcidin response, demonstrating the negative feedback mechanism that iron stores have on hepcidin expression to enable effective iron absorption and utilisation within the body when an individual is ID. Therefore in our results the lack of significant difference in hepcidin concentrations for those participants that were iron insufficient is understandable.[13]

Research in 18 to 25-year-old overweight Australian females (BMI ≥ 27.5 $kg \cdot m^{-2}$) has suggested that although increasing obesity was associated with minor disturbances in iron metabolism (lower serum iron, lower transferrin saturation, higher Sf and higher CRP), the effect of inflammation and hepcidin alone was not enough to induce significant changes to iron status.[23] Therefore, other factors that are likely to influence iron status need to be considered. In our study, South Asians had 5.28 higher odds of being vegetarian than NZ Europeans, and 4.86 higher odds than those of other ethnicities.[24] As a result, South Asian participants consumed meat less frequently (3.5 times per week) than NZ Europeans (8.33 times per week) and those of other ethnicities (11.25 times per week).[24] This aligns with findings from the most recent National Nutrition Survey, with South Asian females being the most likely ethnic group to have never eaten chicken, red meat and processed meat.[24]

A low dietary haem intake has consistently been identified as a risk factor for ID, likely due to haem iron being more easily absorbed than non-haem iron.[9,24,41,42] In Dunedin pre-menopausal females, consuming meat/fish/poultry intake >79 g per day was found to be protective against mild ID (Sf <20 $\mu g \cdot L^{-1}$).[9] While a study in Auckland premenopausal females with children found that those following a 'meat and vegetable' dietary pattern had 0.21 reduced odds of having ID (Sf <20 $\mu g \cdot L^{-1}$).[3]

The amalgamation of risk factors for ID found in South Asian participants (increased body fat, increased inflammation, increased hepcidin levels) highlight this ethnic group as potentially having an increased risk of being unable to absorb and utilise iron over time. This physiological state that would appear to be unsupportive of iron absorption and utilisation in combination a low dietary haem intake would suggest that South Asian females are at a higher risk of developing ID as compared to other ethnicities within New Zealand.

Overall predictors of iron deficiency

Using multiple linear regression analysis, our final model showed that hepcidin and frequency of meat intake were the only significant predictors of Sf in our study population. Meat intake was only correlated with Sf in NZ European participants, whereas hepcidin was a predictor of Sf in all ethnicities.

A positive correlation between Sf and serum hepcidin was also found in Dutch males and females, with a 1% increase in Sf associated with a 0.81-0.85% increase in serum hepcidin.[39] A study in Australian premenopausal females demonstrated that a 10% increase in red and white meat intake corresponded with a 0.9% increase in Sf, however, they too found hepcidin levels were a stronger predictor of iron stores,[43] thus reinforcing the findings from this study.

The high levels of hepcidin in iron sufficient South Asians could also explain why this ethnic group had a higher rate of ID, although not significant. Hepcidin's effect on regulating dietary haem absorption is still an emerging area of research, however, in rats injected with hepcidin, significantly reduced mucosal iron uptake was observed.[44] Therefore, supporting our previous statement that South Asian participants may have altered iron absorption due to elevated hepcidin levels and in combination with a low meat intake may have an increased risk of developing ID.

Conclusion

Our cohort had a high rate of ID compared to previous research undertaken in NZ premenopausal females. This is likely explained by a high representation of South Asian participants as well as the high rates of vegetarianism in our cohort, a rising trend amongst young females.

South Asians were more likely to have a higher BMI, body fat percentage and IL-6 as compared to NZ Europeans and those of other ethnicities. These factors could be possible explanations for iron sufficient South Asian females having a higher serum hepcidin concentration than NZ Europeans and those of other ethnicities. These results would suggest that South Asians are an at-risk group for ID development.

Hepcidin and frequency of meat intake were significant predictors of ID in NZ Europeans, with hepcidin being

the stronger predictor. Health professionals should continue to be vigilant around the rise in meat restriction as a risk factor for ID, particularly in premenopausal females, as this group is among the most likely to restrict their meat intake. Those of South Asian ethnicities should also be highlighted as an at-risk group, and iron status of these individuals should be monitored regularly. The strong correlation of hepcidin with Sf aligns with previous research and continues to highlight hepcidin as an emerging biomarker for identifying ID.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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REFERENCES

1. World Health Organisation. The global prevalence of anemia in 2011. Geneva: WHO; 2011.
2. University of Otago, Ministry of Health. A Focus on Nutrition: Key Findings of the 2008/09 New Zealand Adult Nutrition Survey. 2011 [cited 2019/02/16]; Available from https://www.health.govt.nz/publication/focus-nutrition-key-findings-2008-09-nz-adult-nutrition-survey.
3. Beck KL, Conlon CA, Kruger R, Heath A-LM, Matthys C, Coad J et al. Blood donation, being Asian, and a history of iron deficiency are stronger predictors of iron deficiency than dietary patterns in premenopausal women. BioMed Res Int. 2014;2014:652860. doi: 10.1155/2014/652860.
4. Wall CR, Brunt DR, Grant CC. Ethnic variance in iron status: is it related to dietary intake? Public Health Nutr. 2009;12:1413-21. doi: 10.1017/S1368980008004187.
5. Hunt JR, Roughead ZK. Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. Am J Clin Nutr. 1999;69:944-52. doi: 10.1093/ajcn/69.5.944.
6. Roy Morgan Research. Vegetarianism on the rise in New Zealand. 2016 [cited 2018/10/14]; Available from http://www.roymorgan.com/findings/6663-vegetarians-on-the-rise-in-new-zealand-june-2015-201602080028.
7. Woolf A-L. The rise of vegetarians: 1 in 10 New Zealanders mostly, or completely, meat-free. Stuff. 2019 [cited 2019/2/6]. Available from https://www.stuff.co.nz/environment/110495348/one-in-ten-new-zealanders-mostly-or-completely-meatfree.
8. Monsen ER, Hallberg L, Layrisse M, Hegsted DM, Cook JD, Mertz W et al. Estimation of available dietary iron. Am J Clin Nutr. 1978;31:134-41. doi: 10.1093/ajcn/31.1.134.
9. Heath AL, 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. Public Health Nutr. 2001;4:197-206. doi: 10.1079/PHN2000 54.
10. Napolitano M, Dolce A, Celenza G, Grandone E, Perilli MG, Siragusa S et al. Iron-dependent erythropoiesis in women with excessive menstrual blood losses and women with normal menses. Ann Hematol. 2014;93:557-63. doi: 10.1007/s00277-013-1901-3.
11. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. Lancet. 2007;370:511-20. doi: 10.1016/S0140-6736(07)61235-5.
12. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 2004;306:2090-3.
13. Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011;117:4425-33 doi: 10.1182/blood-2011-01-258467.
14. Leong W-I, Lönnerdal B. Hepcidin, the recently identified peptide that appears to regulate iron absorption. J Nutr. 2004;134:1-4. doi: 10.1093/jn/134.1.1.
15. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest. 2004;113:1271-6.
16. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood. 2008;112:4292-7. doi: 10.1182/blood-2008-02-139915.
17. Ilkovska B, Kotevska B, Trifunov G, Kanazirev B. Serum hepcidin reference range, gender differences, menopausal dependence and biochemical correlates in healthy subjects. J IMAB-Annual Proceeding. 2016;22:1127-31. doi: 10.5272/jimab.2016222.1127.
18. Verga Falzacappa MV, Vujic Spasic M, Kessler R, Stolte J, Hentze MW, Muckenthaler MU. STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. Blood. 2007;109:353.
19. Andrews NC. Anemia of inflammation: the cytokine-hepcidin link. J Clin Invest. 2004;113:1251-3. doi: 10.1172/JCI21441.
20. Ganz T, Enns CA. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood. 2003;102:783-8 doi: 10.1182/blood-2003-03-0672.
21. Aigner E, Feldman A, Datz C. Obesity as an emerging risk factor for iron deficiency. Nutrients. 2014;6:3587-600. doi: 10.3390/nu6093587.
22. Ministry of Health. Obesity Statistics. 2017; [Cited 2019/02/17]. Available from: https://www.health.govt.nz/nz-health-statistics/health-statistics-and-data-sets/obesity-statistics
23. Cheng HL, Bryant CE, Rooney KB, Steinbeck KS, Griffin HJ, Petocz P et al. Iron, hepcidin and inflammatory status of young healthy overweight and obese women in Australia. PLoS One. 2013;8:e68675. doi: 10.1371/journal.pone.0068675.
24. Parackal SM, Smith C, Parnell WR. A profile of New Zealand 'Asian' participants of the 2008/09 Adult National Nutrition Survey: focus on dietary habits, nutrient intakes and health outcomes. Public Health Nutr. 2015;18:893-904. doi: 10.1017/S1368980014001049.
25. Gotloib L, Silverberg D, Fudin R, Shostak A. Iron deficiency is a common cause of anemia in chronic kidney disease and can often be corrected with intravenous iron. J Nephrol. 2006;19:161-7.
26. Gasche C, Lomer MCE, Cavill I, Weiss G. Iron, anaemia, and inflammatory bowel diseases. Gut. 2004;53:1190-7. doi: 10.1136/gut.2003.035758.
27. R&D Systems. Human Hepcidin Quantikine ELISA Kit DHP250. 2019 [cited 2019/11/23]; Available from: https://www.rndsystems.com/products/human-hepcidin-quantikine-elisa-kit_dhp250
28. R&D Systems. Human IL-6 Quantikine ELISA Kit D6050 [Internet]. 2019 [cited 2019/11/23]; Available from: https://www.rndsystems.com/products/human-il-6-quantikine-elisa-kit_d6050
29. Beck KL, Conlon CA, Kruger R, Heath AL, Matthys C, Coad J, et al. Iron status and self-perceived health, well-being, and fatigue in female university students living in New Zealand. J Am Coll Nutr. 2012;31:45-53.
30. Heath ALM, Skeaff CM, Gibson RS. Validation of a questionnaire method for estimating extent of menstrual blood loss in young adult women. J Trace Elem Med Biol. 1999;12:231-5. doi: 10.1016/S0946-672X(99)80063-7.

31. Bui VQ, Stein AD, DiGirolamo AM, Ramakrishnan U, Flores-Ayala RC, Ramirez-Zea M et al. Associations between serum C-reactive protein and serum zinc, ferritin, and copper in Guatemalan school children. Biol Trace Elem Res. 2012;148:154. doi: 10.1007/S12011-012-9358-0.
32. Nemeth E, Ganz T. The role of hepcidin in iron metabolism. Acta Haematol. 2009;122:78-86. doi: 10.1159/000243791.
33. Krishnaveni G V, Hill JC, Veena SR, Leary SD, Saperia J, Chachyamma KJ et al. Truncal adiposity is present at birth and in early childhood in South Indian children. Indian Pediatr. 2005;42:527-38.
34. Rush EC, Freitas I, Plank LD. Body size, body composition and fat distribution: comparative analysis of European, Maori, Pacific Island and Asian Indian adults. Br J Nutr. 2009;102:632. doi: 10.1017/S0007114508207221.
35. Wulan SN, Westerterp KR, Plasqui G. Ethnic differences in body composition and the associated metabolic profile: A comparative study between Asians and Caucasians. Maturitas. 2010;65:315-9. doi: 10.1016/J.MATURITAS.2009.12.012.
36. Rush EC, Goedecke JH, Jennings C, Micklesfield L, Dugas L, Lambert E V et al. BMI, fat and muscle differences in urban women of five ethnicities from two countries. Int J Obes. 2007;31:1232-9. doi: 10.1038/sj.ijo.0803576.
37. Peters MJL, Ghouri N, McKeigue P, Forouhi NG, Sattar N. Circulating IL-6 concentrations and associated anthropometric and metabolic parameters in South Asian men and women in comparison to European whites. Cytokine. 2013;61:29-32. doi: 10.1016/J.CYTO.2012.09.002.
38. Eder K, Baffy N, Falus A, Fulop AK. The major inflammatory mediator interleukin-6 and obesity. Inflamm Res. 2009;58:727-36. doi: 10.1007/s00011-009-0060-4.
39. Galesloot TE, Vermeulen SH, Geurts-Moespot AJ, Klaver SM, Kroot JJ, Van Tienoven D et al. Serum hepcidin: reference ranges and biochemical correlates in the general population. 2011;117:e218-25. doi: 10.1182/blood-2011-02-337907.
40. Tussing-Humphreys LM, Nemeth E, Fantuzzi G, Freels S, Holterman AX, Galvani C et al. Decreased serum hepcidin and improved functional iron status 6 months after restrictive bariatric surgery. Obesity. 2010;18:2010-6. doi: 10.1038/oby.2009.490.
41. Craig WJ. Nutrition concerns and health effects of vegetarian diets. Nutr Clin Pract. 2010;25:613-20. doi: 10.1177/0884533610385707.
42. Hunt JR. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr. 2003;78:633S-9S. doi: 10.1093/ajcn/78.3.633S.
43. Lim K, Booth A, Nowson C, Szymlek-Gay E, Irving D, Riddell L. Hepcidin is a better predictor of iron stores in premenopausal women than blood loss or dietary intake. Nutrients. 2016;8:540. doi: 10.3390/nu8090540.
44. Laftah AH, Ramesh B, Simpson RJ, Solanky N, Bahram S, Schümann K et al. Effect of hepcidin on intestinal iron absorption in mice. Blood. 2004;103:3940-4. doi: 10.1182/blood-2003-03-0953.