J Food Sci Technol (April 2016) 53(4):2001–2008
DOI 10.1007/s13197-015-2153-0

ORIGINAL ARTICLE

Relationship of dietary factors with dialyzable iron and in vitro iron bioavailability in the meals of farm women

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Revised: 24 September 2014 / Accepted: 29 September 2014 / Published online: 15 April 2016

Abstract Sixty rural women with age varying between 25 and 35 were selected randomly to determine the role of dietary factors on bioavailability of iron in their diets. Food samples of selected subjects were collected for three major meals i.e. breakfast, lunch and dinner for three consecutive days. The samples were analyzed for meal constituents associated with iron absorption as well as for total and dialyzable iron. Based on dietary characteristics, the diets of the farm women were in the class of intermediate diets as per FAO/WHO classification with iron bioavailability of 8.11 %. The statistical analysis revealed that the meal constituents which were found to influence iron absorption positively were ascorbic acid and β-carotene in breakfast and only β-carotene in dinner. The meal constituents which affected iron absorption negatively were zinc and calcium in breakfast as well as lunch and phytates and NDF in dinner, however, polyphenols present in the meals of the subjects did not show any relationship with iron absorption.

Keywords Iron bioavailability · Farm women · Iron inhibitors · Iron enhancers · Dialyzable iron

Introduction

Iron deficiency is the most widespread deficiency worldwide. It is estimated that more than two billion people worldwide are iron deficient, including one billion people who have iron deficiency anemia (WHO 2004). In India, 52 % of young women have anemia (NFHS 2000). These women are vulnerable to iron deficiency because of their greater requirements of iron for menstruation and childbearing. Lower iron absorption could be a major mechanism by which nutritional iron deficiency affects populations. The large and expanding list of dietary factors that affect the bioavailability of food iron has made it increasingly difficult to predict iron absorption from complex meals (Carpenter and Mahoney 1992; Hallberg 1981 and Cook et al. 1981). The absorption of non heme iron is influenced by a variety of inhibitors and enhancers of iron absorption. Proteins, sugars, ascorbic acid and citric acid have been shown to enhance iron availability whereas phytic acid, fibre and tannins tend to inhibit it. The absorbed amount of non-heme iron is highly variable and also depends on the individual's iron status. In most parts of the world, including India, wheat is the staple food. Vegetarian diets are mainly plant based, although a vegetarian diet is likely to contain iron in amount equivalent to amount in a non vegetarian diet, the iron from vegetarian diet is likely to be substantially less available for absorption (FNB 2001) because of difference in the chemical form of iron and the accompanying constituent that enhance or inhibit iron absorption (Hunt 2003). Most of Indian population is vegetarian, hence exposed to development of mild to severe degree of anemia. A number of studies on the availability of food iron and related aspects have shown the possibility of improving the bioavailability of iron in plant foods (Allen and Ahluwalia 1997). An in vitro method that stimulates human digestion and absorption of dietary iron from complex meals has been described by Miller et al. (1981). Both enhancing and inhibiting factors of iron absorption have been shown to respond in vitro in the same way as they affect iron absorption in vivo. A significant correlation was found between iron absorption in vivo and the in vitro method of iron availability assessment.

Iron deficiency commonly associated with plant-based diets in impoverished nations are not associated with vegetarian

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diets in wealthier countries, the nutrients warrant attention as nutritional assessment methods become more sensitive and plant-based diets receive greater emphasis that the iron from vegetarian diets is generally less bioavailable than from non vegetarian diets because of reduced meat intake as well as the tendency to consume more phytic acid and other plant-based inhibitors of iron absorption. Food consumption in India is varied and influenced by regional, ethnic, cultural, income and agricultural production differences (FAO 1998). The cereal consumption pattern in India is diverse as rice, wheat, maize are consumed to varying quantities. According to the recent diet survey in the rural areas of nine states, rice, wheat and millets like *ragi* and sorghum are the widely consumed staple of various regions of India (NNMB 2006). The average daily intake of cereals and millets ranged from 320 to 477 g as against a recommended intake of 460 g, providing about 30–82 % of total dietary non-haem iron from 18 to 37 g and contributes to 5–10 % of dietary intake of iron while that from green leafy vegetable is only 2–5 %. (Nair and Lyengar 2009)

Punjab state has a surplus of food grain production. There is also abundant production of green leafy vegetables in the winter. In spite of adequate food production, iron deficiency continues to be the major nutritional disorder adversely affecting work efficiency of women (Bains and Mann 2000). A survey conducted among rural families of Punjab revealed that the consumption of nutrients like energy, protein, fat, iron, folic acid, and ascorbic acid were adequate. Though the intake of iron was nearly adequate, but the blood hemoglobin level of women indicated that 47 % were anemic with an average hemoglobin level of 10.8 g/dl. The anemia prevalence was high regardless of adequate consumption of iron which is attributed to poor availability of dietary iron from traditional Punjabi diets. (Bains et al. 2006). Looking to the importance of bioavailability of iron and its association with inhibitors and enhancers, the present study was conducted to analyze the relationship between the enhancing or inhibiting factors and in vitro iron bioavailability in the meals of adult farm women of Punjab state of India.

Materials and methods

Selection of subjects To achieve set forth objective of the study, village Ayali Kalan from Ludhiana district of Punjab State, India was selected. The village has a farm community comprising of marginal, small, medium and large farmers. A list of farm women with age between 25 and 35 years from the families of medium farmers with land holdings of 2–4 ha was prepared using the voter list from village Sarpanch. The women (sixty in number) who were non pregnant, non lactating and healthy with no incidence of any major disease during the last 6 months were selected for the study. A rapport building with selected women was made taking the help of key communicators of village (village Sarpanch and members of village panchayat). A prior written consent of the subjects to participate in the study was obtained. The women were explained objective of the study and possible benefits. They gave their consent to become subjects of study with an informal agreement of getting educational advisory for improving bioavailability of iron in their diets.

Health and nutritional survey An interview schedule was developed to collect the information of the subjects such as age, education, occupation, type of family, family income and number of children was collected from the subjects by interview method. '24 h Recall Method' for three consecutive days was used to assess food intake of the subjects. The major nutrients were calculated using MSU Nutriguide Computer Program (Song et al. 1992). The intake of nutrients i.e. energy, protein, β-carotene, folate, ascorbic acid, iron, and calcium in each meal was assessed and percent adequacy was determined by comparing the nutrient intake with recommended dietary allowance (RDA) for that nutrient (ICMR 2010).

Haemoglobin (Hb) of the subjects was determined using the hemoglobin kit supplied by Becan Diagnostic Private Limited. 20 μg of blood was drawn from the third finger of the left hand with a haemoglobin pipette and delivered into a test tube containing 5 ml drabkin's solution. Haemoglobin is converted to cyanomethaemoglobin containing potassium cyanide and potassium ferricyanide which is measured in the colorimeter and Hb is calculated from Standard curve prepared from the standard provided in the kit. The subjects were classified on the basis of their hemoglobin levels based on the anemia classification by WHO (2011).

Food sample collection The duplicate samples of three major meals of the selected subjects were collected in the decontaminated plastic jars for three consecutive days. The samples collected from the subjects were brought to the laboratory of department of Food and Nutrition, Punjab Agricultural University, Ludhiana. The 3 days meals were pooled for each subject and then homogenized using domestic food blender with stainless steel blades. A portion of homogenized sample of each meal was kept in hot air oven at 60 °C till constant weight to determine the moisture content in the fresh samples. Another portion of homogenized samples was stored in zip lock bags at −18 °C for further analysis.

Chemical analysis The dried samples were analyzed for protein, neutral detergent fibre (NDF), phytates, polyphenols and total minerals using standard methods as protein was determined by macrokjeldhal method (AOAC 1990), NDF by AOAC (1990) method, phytin phosphorus by the method given by Haug and Lantzsch (1983) and polyphenols by

AOAC (1985) method. The minerals namely iron, calcium and zinc were estimated by Atomic Absorption Spectrometer after wet digestion. β- carotene was analyzed by column chromatography (Rangana 1995) while ascorbic acid was assessed by the calorimetric method described by AOAC (1996) in fresh samples.

An in vitro method for estimating dialyzable iron involved simulated gastrointestinal digestion followed by measurement of soluble, low molecular weight iron. Meals (20 g) were homogenized and exposed to pepsin at pH 2. Dialysis was used to adjust the pH to intestinal levels and digestion was continued after the addition pancreatin and bile salts. Iron from the digestion mixture which diffused across a 6,000 to 8,000 molecular weight cutoff semi-permeable dialyzable membrane was used as an indicator of available iron (Miller et al.1981). Iron present in the dialysate at the end of simulated gastrointestinal digestion represented the dialyzable iron fraction. Dialysates were mixed with 5 ml of nitric acid and analyzed the dialyzable iron content at Atomic Absorption Spectrophotometer. Bioavailable iron was calculated as: In vitro iron bioavailability (%) = Dialyzable iron/ Total iron x 100.

$$\text{Bioavalilable iron} = \frac{\text{Dialyzable iron}}{\text{Total iron}} \times 100$$

Statistical analysis The results were evaluated by mean, standard deviation and analysis of variance (ANOVA) to check the critical difference. All nutrient values were expressed as the total amounts in each meal. The correlation coefficient between the nutrient which affects iron bioavailability positively or negatively was used to test the influence of meal constituents. All calculation were done on Microsoft excel- 2007.

Result and discussion

Health and nutritional profile of the subjects

Forty percent of the subjects had their hemoglobin tested and only 3 % reported deworming treatment in the last 1 year. The mean hemoglobin of the subjects was 10.5 ± 1.18 g/dl. Eighty three percent of the subjects were anemic out of which 13 and 70 % were found to be mildly and moderately anemic. The overall intake of food groups namely cereals, pulses, green leafy vegetables, roots and tubers, fruits, other vegetables, milk and milk products, sugars and fats and oils by women was 352, 40.3, 4.6, 62.7, 49.0, 15.7, 365, 42.2 and 54.0 g/day, the percent adequacy being 98, 54, 5, 63, 49, 16, 122, 141 and 216 %, respectively. Out of seven nutrients studied, calcium was adequate while five of them namely energy, protein, folate, ascorbic acid and iron were marginally adequate with nutrient adequacy ranged between 75 and 100 %. Only β-carotene was marginally inadequate. Though the intake of most of the nutrients related to iron nutriture of the farm women were marginally adequate, the hematological profile of the subjects as revealed by their hemoglobin levels showed the dismal condition of their iron status.

Analysis of meals for constituents associated with iron absorption

There is hefty and expanding record of meal constituents that influences the bioavailability of food iron. The content of protein, ascorbic acid, β-carotene, phytin phosphorus, polyphenols, NDF, calcium and zinc in representative meals i.e. breakfast, lunch and dinner of the farm women has been shown in Table 1.

The average total protein in three meals namely breakfast, lunch and dinner ranged between 7.5 to 22.3, 7.3 to 24.1 and 10.6 to 41.2 g with the mean values of 14.9, 12.7 and 18.0 g, respectively. The total average protein in three meals ranged between 29.8 and 87.7 g with the mean value of 45.6 g. Kaur (1999) found that the average daily intake of protein among the adult farm women varied between 35.7 and 53.6 g with the mean intake of 43.3 g. the mean protein intake was 87 % of the Recommended Dietary Allowances (RDA). Kaur (2012) reported that the daily average intake of protein by the subjects was 52.7 g. In another study, Bains et al. (2012) found the protein intake of women as 48.6 g by rural adult women. There is good evidence to support the enhancing effect of cysteine-containing peptides (Layrisse et al. 1984 and Taylor et al. 1986), which are rich in digests of myofibrillar proteins and which, like ascorbic acid, could both reduce and chelate iron. Storcksdieck et al. (2007). The proteins in the diets of the subjects in the present study were totally derived from either plant sources or milk and milk products. The consumption of flesh foods such as meat, chicken, fish etc. which promotes iron absorption was found to be negligible. Hence, protein as meal constituent cannot be considered as the determinant of iron absorption in the meals of the selected subjects.

The daily ascorbic acid content of breakfast, lunch and dinner ranged between 0 to 4, 0 to 6 and 0.2 to 5 mg with the mean value of 1.3, 2.1 and 2.0 mg, respectively. Statistical difference in ascorbic acid content of three different meals was found to be non significant. The total ascorbic acid in three meals was 2.4 to 9.0 mg with mean value of 5.5 mg. The low level of ascorbic acid in the meals of the subjects by the farm women may influence the iron absorption negatively. Several literature reports highlighted the positive role of ascorbic acid in improving iron status of women pertaining to its role in iron absorption (Seshadri 1993; Nayak and Nair 2003 and Fidler et al. 2004). A significant increase in hemoglobin levels were

Table 1 Analysis of meals of farm women for constituents associated with iron absorption

Meal Constituents	Breakfast	Lunch	Dinner	*P* value	C.D. at 5 %	Total
Protein, g						
Range	7.5–22.3	7.3–24.1	10.6–41.2			
Mean ± SD	14.9 ± 4.5	12.7 ± 4.0	18.0 ± 7.9	NS	–	45.6 ± 14.1
Ascorbic Acid, mg						
Range	0–4	0–6	0.2–5			
Mean ± SD	1.3 ± 1.59	2.1 ± 1.94	2.03 ± 1.33	NS	–	5.5 ± 2.0
β-Carotene, μg						
Range	0–132.0	0–206.3	0–210.3			
Mean ± SD	28.8 ± 49.9	42.7 ± 65.5	69.0 ± 84.6	NS	–	140.6 ± 90.1
Phytin phosphorus, mg						
Range	64.2–138.0	52.5–144.0	76.3–209.0			
Mean ± SD	99.3 ± 22.4	81.0 ± 23.6	114.6 ± 36.0	≤0.01	25.3	294.8 ± 64.2
Polyphenols, mg						
Range	73.9–133.0	52.5–128.0	83.7–209.0			
Mean ± SD	102.0 ± 21.0	74.6 ± 19.8	122.8 ± 33.3	≤0.01	23.0	299.9 ± 56.6
NDF, mg						
Range	3.6–14.0	4.5–15.0	4.8–20.0			
Mean ± SD	8.9 ± 3.2	8.0 ± 3.2	9.7 ± 4.7	NS	–	26.6 ± 8.7
Zinc, mg						
Range	0.5–5.9	0.7–2.3	0.7–3.5			
Mean ± SD	1.7 ± 1.3	1.3 ± 0.4	1.5 ± 0.7	NS	–	4.4 ± 1.8
Calcium, mg						
Range	21.8–289.6	34.6–121.9	29.9–225.6			
Mean ± SD	116.8 ± 71.8	84.0 ± 28.4	113.7 ± 73.8	NS	–	314.5 ± 126.3

**P* value 0.01 – Significant at 1 %; NS – Non significant

† Protein (AOAC 1990); Ascorbic acid (AOAC 1996); β- carotene (Rangana 1995); Phytin phosphorus (Haug and Lantzsch 1983); Polyphenols (AOAC 1985); NDF (AOAC 1990); Iron, calcium and zinc (Atomic Absorption Spectrometer)

observed after an intake of 200 mg ascorbic acid daily for 60 days in anemic children in India (Seshadri et al. 1985). In adults, 500 mg of ascorbic acid given to strict vegetarians improved iron status after 2 months (Sharma and Mathur 1995).

β-carotene value of all the three meals were quite low, the range in breakfast, lunch and dinner being 0 to 132.0, 0 to 206.3 and 0 to 210.3 mg with mean value of 28.8, 42.7 and 69.0 μg, respectively. No significant difference was found in β-carotene of the three meals. The overall intake of β-carotene from three meals ranged between 0 and 210.3 μg with a mean value of 140.6 μg. Nagi et al. (1995) reported a very low intake of β-carotene as compared to RDA in adolescent girls of Ludhiana city. Vitamin A deficiency such as iron deficiency leads to anemia. Vitamin A can affect several stages of iron metabolism (Hurrell 2004), which include erythropoiesis and the release of iron from ferritin stores (Zimmermann et al. 2006). Recent studies suggest that vitamin A and β-carotene can enhance non-haem iron absorption and thereby contribute to an increase in hemoglobin levels (García-Casal et al. 1998).

Phytin phosphorus as one of the inhibitor of iron absorption ranged between 64.2 to 138.0, 52.5 to 144.0 and 76.3 to 209.0 mg in breakfast, lunch and dinner with the mean value of 99.3, 81.0 and 114.6 mg, respectively. Dinner had significantly ($p \leq 0.01$) higher phytin phosphorus as compared to lunch. The reason for lower values was that lunch was a smaller meal as compared to dinner. The phytin phosphorus was mainly contributed by the whole wheat flour and whole legumes which were the major foods in the meals of the subjects from whom food samples were collected. The high phytin phosphorus content of the meals could influence iron absorption negatively. The daily total phytin phosphorus ranged between 201.1 to 352.0 mg with a mean value of 294.8 mg. Sharma (2003) reported that Indian diet is mainly non-heme iron diet. The absorption of non-heme iron is controlled by many food components, one is phytate in grains, legumes, nuts, vegetables, roots and tubers and fruits which can decrease non haem iron absorption by 51.8 %. Grewal (1992) found that the phytin phosphorus content of daily diets of women in the range of 181 to 215 mg and were higher than the values observed in the present study. Reddy (2002) reported that the daily intake of phytate for humans on vegetarian diet, on an average is 2,000 mg, 2,600 mg whilst, for inhabitants of rural areas in developing countries on mixed diets, it is 150–1,400 mg. Higher levels of phytates in the diets have negative influence on iron bioavailability as it is reported to form insoluble phytate-mineral complexes leading to decrease in mineral availability. (Lopez et al. 2002 and Konietzny and Greiner 2003).

Polyphenols occur in various amounts in plant foods and beverages, such as vegetables, fruits, some cereals and legumes, tea, coffee, and wine. There was a high content of polyphenols in breakfast and dinner. The range of polyphenols in breakfast, lunch and dinner was 73.9 to 133.0, 52.5 to 128.0 and 83.7 to 209.0 mg, with the mean value of 102.0, 74.6 and 122.8 mg, respectively. Dinner had significantly ($p \leq 0.01$) higher polyphenol content as compared to lunch. The reason for lower values was that a lunch was smaller meal as compared to dinner. The presence of whole pulses in dinner may contribute to high polyphenols in dinner. The average total daily polyphenol intake ranged between 229.0 to 453 mg with an average value of 299.9 mg. Grewal (1992) found intake of 825, 966, 1,164 mg per day by low, middle and high income group women from Ludhiana. In cereals and legumes, polyphenols add to the inhibitory effect of phytate, as was shown in a study that compared high and low polyphenol sorghum. After complete phytate degradation, iron absorption from low-polyphenol sorghum increased significantly, whereas iron absorption from high-polyphenol sorghum was not improved (Hurrell 2004).

The average total NDF which is mainly composed of cellulose and hemicellulose ranged between 3.6 to 14.0, 4.5 to 15.0 and 4.8 to 20.0 g with the mean value of 8.9, 8.0 and 9.7 g in three meals, respectively. The total average neutral detergent fiber ranged between 16.3 and 50 g with the mean value of 26.6 mg. No significant difference in NDF values was found between the three meals. Grewal (1992) found that the intake of NDF was 31.0 to 38.6 g per day by women belonging to different socio-economic groups. The values obtained in the present study are lower then the values of the reported study. Another study conducted by Kochar (1981) for analyzing the effect of dietary fiber on the utilization of various nutrients showed that absorption of minerals like iron, calcium and zinc decreased in fiber supplemented groups. This may be due to physio-chemical effects resulting from increased bulk and properties of fiber such as water binding which reduce the transit time and diffusion rate of the digested products towards the absorptive mucosal surfaces etc.

Calcium element is reported to be significant competitors of iron absorption hence if present in appreciable amount in the meals may lower the bioavailability of iron. The studied community consumed fairly good amount of milk and milk products, hence calcium intake from three meals was sufficient. The calcium in the collected three meals i.e. breakfast, lunch and dinner ranged between 21.8 to 289.6, 34.6 to121.9 and 29.9 to 225.6 mg with the mean values of 116.8, 84.0 and 113.7 mg, respectively. The average daily calcium intake from three meals ranged between 109.4 and 490.7 mg with an average value of 314.5 mg. Chaudhary (2012), Kushwaha (2011) and Chandla (2006) reported a higher intake of calcium by Punjabi women. Calcium is an important nutrient, hence should be included in the diet for optimum health. Practical solutions for the competition of calcium with iron is to increase iron intake, increase its bioavailability or avoid taking calcium and iron-rich foods at the same time.

The daily intake of zinc from three major meals ranged between 0.47 to 5.91, 0.74 to 2.29 and 0.73 to3.53 mg with the mean values of 1.7, 1.3 and 1.5 mg, respectively. The average daily zinc intake ranged between 2.2 to 8.8 mg with an average value of 4.4 mg. Bains et al. (2012) reported that the mean value of zinc intake as 5.98 mg. Zinc is a vital nutrient which plays a significant role in maintaining health, however being a divalent along with calcium play major role in reducing absorption of iron in the mixed meals. Iron and zinc deficiencies may occur simultaneously. Experimental animal studies indicate that iron and zinc are essential components of the brain, involved in its development and in central nervous system functions. High iron intake can reduce zinc levels and high zinc intake can reduce iron absorption and encourage iron depletion from body stores. Low consumption of foods rich in bioavailable iron and zinc such as meat, particularly red meat, and high consumption of foods rich in inhibitors of iron and zinc absorption, such as phytate, certain dietary fibers and calcium cause iron and zinc deficiencies.

Analysis of meals for total iron, dialyzable iron and in vitro iron bioavailability

The results presented in Table 2 revealed that the average iron content ranged from 1.03 to 4.14 mg in breakfast, 0.99 to 8.85 mg in lunch and 0.50 to 9.67 mg in dinner. The average intake of iron in breakfast, lunch and dinner was 2.31, 2.66 and 3.10 mg, respectively. The total iron intake by the subjects from all the meals was 8.07 mg/day. Nair and Lyengar (2009) reported that the intake of iron in India was less than 50 % of the recommended dietary allowance and iron density was about 8.5 mg /1,000 kcal. Jain (2009) reported low intake of iron by Punjabi women i.e. 17 mg/day which almost double of the present study. Kim et al. (2007) reported even much lower intake of iron by both normal and osteoporotic women. Findings of present study were in line with the findings of Sandhu (1998), Singh and Aggarwal (2001), Shoker (2003), and Gungeet (2004), who reported inadequate intake of iron by the Punjabi women.

Table 2 Analysis of meals of farm women for total iron, dialyzable iron and in vitro iron bioavailability

Parameter	Meal			*P* value	CD at 5 %	Total
	Breakfast	Lunch	Dinner			
Total iron, mg						
Range	1.03–4.14	0.99–8.85	0.50–9.67			
Mean ± SD	2.31 ± 1.04	2.66 ± 1.99	3.10 ± 2.11	NS	–	8.07 ± 3.75
Dialyzable iron, mg @						
Range	0.12–0.30	0.11–0.33	0.01–0.90			
Mean ± SD	0.17 ± 0.06	0.18 ± 0.07	0.28 ± 0.21	NS	–	0.63 ± 0.25
In vitro iron bioavailability, %						
Range	5.02–11.62	3.73–11.14	2.01–12.49			
Mean ± SD	7.92 ± 1.64	7.54 ± 2.01	8.86 ± 3.10	NS	–	8.11 ± 1.34[a]

P value-0.01significant at 1 %

[a] Mean value of breakfast, lunch and dinner

†Dialyzable iron (Miller 1981)

The average dialyzable iron in analyzed meals namely breakfast, lunch and dinner ranged from 0.12 to 0.30, 0.11 to 0.33 and 0.01 to 0.90 mg with the mean values of 0.17, 0.18 and 0.28 mg, respectively. The average intake of dialyzable iron was maximum in dinner (0.28 mg) followed by breakfast (0.17 mg) and lunch (0.18 mg). The total dialyzable iron intake from three meals was 0.63 mg by the farm women.

The maximum bioavailability of iron was in dinner followed by breakfast and lunch. The in vitro iron bioavailability ranged from 5.02 to 11.62, 3.73 to 11.14 and 2.01 to 12.49 in breakfast, lunch and dinner with the mean values of 7.92, 7.54 and 8.86 %, respectively. Based on the classification of FAO/WHO (Zimmermann and Hurrell 2007), the diets of the subjects were in the class of intermediate diets with iron bioavailability ranged between 5 and 10 %. Kalaivani (2009) reported that inadequate dietary iron and poor bioavailability of dietary iron from fiber and phytate rich Indian diets are the major factors responsible for high prevalence of anemia.

Relationship of meal constituents with dialyzable iron and in vitro iron bioavailability

The relationship of meal constituents with dialyzable iron and in vitro iron bioavailability in each meal has been derived separately as the meal composition for all the three meals was different (Table 3). Two meal constituents namely ascorbic acid and β-carotene showed a positive and significant ($p \leq 0.01$ and 0.1) correlation while zinc showed negative but significant ($p \leq 0.01$) correlation with dialyzable iron in the breakfast. On the other hand, NDF and calcium had negative but significant ($p \leq 0.01$) relation with in vitro iron bioavailability. Ascorbic acid is the most potent enhancer of non-haem iron absorption even in the presence of inhibitors such as phytates, tannates and calcium. It can reduce food

Table 3 Relationship of meal constituents with dialyzable iron and in vitro iron bioavailability in breakfast of the subjects

Dietary factors	Dialyzable iron	In vitro iron bioavailability
Breakfast		
Ascorbic acid	0.50[c]	0.11
β- carotene	0.34[a]	0.12
Phytin phosphorus	−0.11	−0.01
Polyphenols	−0.14	−0.08
Neutral Detergent Fibre	−0.01	−0.62[c]
Zinc	−0.38[b]	−0.12
Calcium	−0.03	−0.35[b]
Lunch		
Ascorbic acid	0.20	0.23
β-carotene	0.29	0.21
Phytin phosphorus	−0.22	−0.10
Polyphenols	−0.29	−0.08
Neutral Detergent Fibre	−0.26	−0.25
Zinc	−0.56[c]	−0.32[a]
Calcium	−0.62[c]	−0.44[b]
Dinner		
Ascorbic acid	0.14	0.23
β-carotene	0.25	0.18[c]
Phytin phosphorus	−0.41[b]	−0.27
Polyphenols	−0.22	−0.27
Neutral Detergent Fibre	−0.09	−0.36[b]
Zinc	−0.37[b]	−0.22

Values are correlation coefficients (r)

[a] Significant at 10 %

[b] Significant at 5 %

[c] Significant at 1 %

ferric iron to the better absorbed ferrous iron by 75–98 %. In Indian studies, the addition of ascorbic acid to cereals and pulses enhanced the available iron (NIN 1992). β-carotene can also enhance non-haem iron absorption and thereby contribute to an increase in hemoglobin levels (García-Casal et al. 1998). The meal constituents which were found to have negative but significant ($p \leq 0.01$) relationship with dialyzable iron were zinc and calcium in lunch. Both the bivalents also affected the in vitro iron bioavailability negatively but significantly ($p \leq 0.01$ and 0.05). An absorption depressing effect of calcium on iron absorption has been demonstrated (Hallberg and Rossander 1991). The inhibitory effect was suggested as occurring during the transport of iron across the basolateral membrane from the enterocyte to the plasma because absorption of both forms of iron is equally inhibited (Hallberg et al. 1992 and Roughead et al. 2005). An addition of 150 mg of calcium to bread or a hamburger meal reduced iron absorption by 50 %. The interaction is suggested to take place within the mucosal cells as absorption of both haem and non-haem iron is affected (Hallberg et al. 1993). In dinner, β-carotene was the only meal constituent which had a positive and significant ($p \leq 0.01$) relationship with in vitro iron bioavailability. Phytin phosphorous and zinc was negatively and significantly ($p \leq 0.01$) correlated with dialyzable iron however, NDF was negatively but significantly ($p \leq 0.01$) correlated with in vitro iron bioavailability. Sodium phytate has been reported to have such inhibitory effect on iron. If bran is used to increase the dietary fiber intake, it would interfere with the absorption of iron due to its phytate content. Kumari (1979) observed that absorption in the low fiber with added cellulose diet and in high fiber diet decreased when compared to low fiber diet. The decreased absorption of iron may be due to the binding of the element to the fiber in the small intestine and secondly due to the decreased intestinal transit time.

Conclusion

It has been concluded that the diets of farm women did not have much of total iron. The bioavailability of iron was intermediate with a mean value lying between 5 and 10 % which was mainly due to higher content of inhibitory factors of iron absorption in their diets. There was also a very little amount of ascorbic acid and β-carotene present in the meals which have been recognized as enhancers of iron bioavailability. The analysis of meals of farm women revealed that the meal constituents which influenced iron absorption positively were ascorbic acid and β-carotene in breakfast and only β-carotene in dinner. The meal constituents which affected iron absorption negatively were zinc and calcium in breakfast as well as lunch and phytates and NDF in dinner. However, polyphenols present in the meals of the subjects did not show any relationship with iron absorption.

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