

Absorption of Non Heme Iron in Typical and Standard Meals using Extrinsically Labeled Iron ⁵⁹Fe

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Abstract

Objective: To study the bioavailability of nonheme iron from standard and test meals in Pakistani adults and to determine iron absorption using extrinsically tagged iron ⁵⁹Fe.

Subjects and Methods: Nonheme iron absorption was measured from standard and test meals in ten healthy individuals. Total calories, carbohydrates, proteins, fats, ascorbic acid, total iron, phytate and ascorbic acid content were determined in both meals. Retention of iron was detected by whole body counting using gamma counter before and after administration of standard, test meals and reference dose.

Results: Iron absorption with test meal was 13% and after adjustment with serum ferritin and reference dose was 16.5% and 14% respectively. The absorption of standard meal was 6.7% which after adjustment with serum ferritin and reference dose was 8.8% and 6.9% respectively. The iron and ascorbic acid content of test meal was 6.5 mg and 5.7 mg respectively while phytate phosphorus content was 114 mg. The iron and ascorbic acid content of standard meal was 1.3 mg and 2.4 mg respectively while phytate phosphorus content was 137 mg.

Conclusion: This evaluated absorption from one of the typical Pakistani diet compared to the standard meal was better. This shows that there are some other physiological factors that lead to iron deficiency anemia in Pakistan (JPMA 54:244;2004).

Introduction

Although iron deficiency is a major nutritional problem in developing countries, it is not generally due to low intake but poor availability of dietary iron. In countries where iron deficiency is prevalent, the intake of heme iron is often negligible and the non-heme iron absorption is very low. Iron deficiency results when the amount of iron absorbed from the diet cannot meet the individual's requirements. Limited absorption may be due to low iron content of the diet or the nature of the diet is such that the iron present in the foods is not easily available for absorption. In developed countries, the iron content of the diet is usually low, about 6 mg/1000 calories, but the dietary iron is relatively available and between 20% and 50% can be absorbed by the iron deficient subjects.¹ This may be ascribed to the high intake of meat, fish and poultry which may both be a good source of iron and enhance the non heme iron absorption.² In many developing countries, however, where diets are largely made up of cereals, the iron content may be relatively high, since the intrinsic iron present in the food is often fortuitously contaminated with soil iron. Although the iron content of such diets may be high, it is usually poorly absorbed. Isotopic studies have shown that iron absorption is inhibited by a number of substances present in such diets, including phytates, calcium phosphate and tannins, in tea.³ When a readily absorbed iron salt is mixed with a meal it forms a common pool with the intrinsic non-heme dietary iron and its absorption is influenced in the same way by inhibiting or enhancing factors present in the diet.⁴ This has important implications when setting goals for fortifying a

diet.

The present study was aimed at determining the bioavailability of non heme iron and absorption of iron from diet commonly consumed by people of Pakistan using extrinsically tagged iron ⁵⁹Fe.

Subjects and Methods

The participants were ten adults with mean age of 40 years (range between 25 to 50 years). They were recruited through public advertising and were selected after an interview and determination of hemoglobin and ferritin levels. They had no apparent underlying disease, had not donated blood or used iron supplements exceeding 20-mg/dl for \geq six months prior to the study and had serum ferritin values ≥ 15 and ≤ 450 μ g/l. They agreed to discontinue all nutritional supplements 6 to 12 weeks before the beginning of the study. None of them routinely used any medications. The participants gave informed consent. The study was approved by University Ethical Committee and Radioactive Drug Research Committee.

Protocol

Subjects consumed weighed diets. Blood indices i.e., hemoglobin, serum iron, total iron binding capacity (TIBC), serum ferritin (ELISA ES 300) and hematological parameters (Medionic cell analyzer CA530) were assessed at day one, before the start of the study. The diets were extrinsically tagged with ⁵⁹Fe. Standard meal was administered on day one, test meal on day fourteen and reference dose on day twenty eight.

Diets

Experimental diets were planned by registered dietician using ordinary food. Standard meal comprised farina flour (40 g), water (250 ml), salt (0.5g), milk (120 ml), butter (14 g), sugar (24 g) and iron (3.92 mg as ferrous sulfate). Ingredients used in test meal were rice and lentils in ratio of 3:1. Reference dose contained ascorbic acid and iron (in 2:1 molar ratio) dissolved in 50 ml of water. The iron content was 3.92 mg as ferrous sulfate and ascorbic acid (18.9 mg) in 50 ml water. The meals were extrinsically tagged with ^{59}Fe in the form of ferric chloride.

All diet ingredients were weighed, prepared and provided to the volunteers by the research center. Foods were weighed and consumed quantitatively. 400 g of standard meal and 250 g of test meal was consumed by each volunteer.

Iron absorption measurements

Absorption of non heme iron was measured by whole body counting after feeding diets extrinsically labeled with ^{59}Fe by Gamma Counter (orbiter 820-824796) in supine and prone positions. The percentage of nonheme iron absorption was measured as the portion of initial whole body activity that remained after 15 days of feeding diets with ^{59}Fe with correction for physical decay and background activity measured before the meals. Each volunteer was called after an overnight fast (8-9-hours) and standard or test meal was given. Only water was allowed for 3 hours following the feeding. Each volunteer was called four times i.e. on day one, after fourteen, twenty-eight and forty two days.

Day one

The volunteers received standard meal (400 g). The meal consisted of 3.92mg of iron as ferrous sulfate extrinsically tagged with 10 microcurie $^{59}\text{FeCl}_3$. Background radioactivity was measured by whole body counter before meal. Whole body counting was done on each volunteer in supine and prone positions, one hour after ingestion of meal.

Day Fourteen

Each volunteer ingested the test meal which consisted of 3.92mg of iron as ferrous sulfate and extrinsically tagged with ^{59}Fe . Whole body counting was done to measure the retention of radioactivity in the volunteers in both supine and prone positions before meal and then one hour after meal.

Day twenty-eight

Each volunteer ingested solution of ascorbic acid and iron (2:1 molar ratio), that contained 3.92mg of iron as ferrous sulfate which was extrinsically tagged with ^{59}Fe

and 18.9mg of ascorbic acid. Whole body counting was done to measure the retention of radioactivity in the volunteers in both supine and prone positions before meal and then one hour after meal.

Day forty-two

Volunteers were called after fourteen days of ingestion of solution. Whole body counting was done to measure the retention of radioactivity in the volunteers in both supine and prone positions.

Standard meal was given 400g and test meal 250g to adjust for the caloric intake by the volunteers.

Chemical Analysis

The food samples that were used as standard meal and test meal in iron absorption studies were analyzed for the carbohydrate, proteins, fats and moisture content by methods of AOAC.⁵ Ascorbic acid was determined by macerating the sample with a stabilizing agent such as 5% metaphosphoric acid and titrating the decanted or filtered extract with 2, 6 dichloroindophenol. Iron was estimated by atomic absorption spectrophotometer⁶, equipped with a graphite furnace and auto sampler. Argon is used as an inert purging gas, the flow is interrupted during the atomization step. Phytate phosphorus contents were determined by the method of Latta and Eskin.⁷ Phytate was extracted directly with hydrochloric acid and then eluted through anion ion exchange resin to separate inorganic phosphorus. The eluted phytate was measured based on interaction between ferric chloride and sulfosalicylic acid (Wade reagent).

Statistics

Data on iron absorption and serum ferritin were logarithmically transformed and then retransformed to obtain original values. Geometric mean are reported. T test was applied to compare the results of the two meals

Results

The study comprises of ten subjects aged 25-50 years. The subjects were administered standard meal, test meal and reference dose extrinsically tagged with radioactive iron (^{59}Fe). Table 1 shows iron absorption in percentage and serum ferritin concentrations. Iron absorption of individual subjects are also shown after adjustment with serum ferritin by formula $\ln A_c = \ln A_o + \ln F_o - \ln 24.5$ (where $\ln A_c$ is logarithm of corrected absorption, A_o is observed absorption, F_o is observed serum ferritin and 24.5 is the geometric mean of serum ferritins). Iron absorption in percentage of individual subjects after adjustment with reference dose by formula $40/R$ is shown in Table 1.

The absorption of test meal and reference dose was significant compared with standard meal when adjusted

Table 1. Iron absorption (% of dose).

The values are adjusted to serum ferritin by the formula $\ln A_c = \ln A_o - \ln F_o - \ln 24.5$ [geometric mean of serum ferritin].

The values are adjusted to Reference Dose by the formula $401 \text{ Reference dose}$.

S.No.	Name of subject	Observed values (x)				Values adjusted with serum ferritin			Values adjusted with reference dose	
		Standard Meal ⁵⁹ Fe %	Test Meal ⁵⁹ Fe %	Reference Dose ⁵⁹ Fe %	Serum Ferritin µg/dl	Standard Meal ⁵⁹ Fe %	Test Meal ⁵⁹ Fe %	Reference Dose ⁵⁹ Fe %	Standard Meal ⁵⁹ Fe %	Test meal Meal ⁵⁹ Fe %
1	K.K	2.0	4.2	57.9	40.0	3.3	6.8	94.5	19	3.7
2	R.S	11.8	25.8	50.8	16.0	7.7	16.8	33.2	10.2	21.2
3	Z.F	6.2	9.9	48.9	15.0	3.8	6.1	29.9	5.6	8.8
4	M.E	7.8	11.9	50.1	36.0	11.5	17.5	74.0	6.9	10.3
5	S.K	14.1	18.5	46.1	35.0	20.1	26.4	65.9	12.8	16.6
6	M.D	4.4	1.4	44.0	16.0	2.9	9.4	28.7	4.2	13.5
7	C.B	8.1	13.5	55.9	15.0	5.0	8.3	34.2	6.8	10.9
8	S.A	2.5	19.8	27.9	22.0	2.2	17.8	25.1	2.8	27.4
9	Y.K	6.6	9.5	35.8	30.0	8.1	11.6	43.8	7.0	10.2
10	S.S	16.0	29.9	77.1	36.0	23.5	43.9	113.3	10.5	17.9
Range		2.0-16.0	4.2-29.9	27.9-77.1	15.0-40.0	2.2-23.5	6.1-43.9	25.1-113.3	1.9-12.8	3.7-27.4
G. mean		6.7	13.0*	49.4*	24.5	8.8	16.5*	54.2*	6.9	14.0*
SEM		0.56	0.60	0.40	0.47	2.34	3.59	9.69	1.4	1.9

*P <0.01 Significant compared with standard meal

AC = Corrected absorption

Fo = Observed ferritin

Ao = Observed absorption

R = Reference dose

Table 2. Composition of meals used in iron absorption studies.

	Carbohydrates g%	Proteins g%	Lipids g%	Moisture content g%
Standard meal (400g)	63.4	17.3	18	1.2
Test meal (250g)	79.0	10.4	8.6	2.0
	Iron content mg	Phytate content mg	Ascorbic acid mg	Total calories cal
Standard meal (400g)	1.3	137	2.4	1616
Test meal (250g)	6.5	114	5.7	1110

The absorption of test meal and reference dose was significant compared with standard meal when adjusted with reference dose as well as serum ferritin.

The composition of standard meal and test meal is shown in table 2. Correlation of standard meal with test meal and reference dose was significant (Figure 1). Correlation between reference dose and serum ferritin was significant ($P < 0.01$) and between test meal and reference dose was nonsignificant. Nonsignificant correlation was found between standard and test meals with serum ferritin and for test meal and reference dose. Correlation between measured and adjusted nonheme iron absorption of standard and test meals were significant ($P < 0.001$), when adjusted with serum ferritin and the reference dose (Figure 2). The iron and ascorbic acid content of test meal was 6.5 mg and 5.7 mg respectively while phytate phosphorus content was 114 mg. The iron and ascorbic acid content of standard meal was 1.3mg and 2.4mg respectively while phytate phosphorus content was 137mg.

Discussion

This study determined iron absorption from typical Pakistani diet given as test meal and compared with standard meal under similar conditions. Study shows that iron absorption from test meal (kitchri) was better when compared with standard meal. Iron absorption was better with test meal because of higher content of iron and ascorbic acid and less phytate phosphorus as compared to standard meal. Reddy et al.⁸ also showed similar results that iron present in fortified meal enhances iron absorption.

Measured iron absorption from the standard meal was 6.7% (Table 1). The values after correction with reference dose and serum ferritin were 6.9% and 8.8% respectively (Table 1). Study by Reddy and Cook⁹ showed from five different studies, that standard meal (farina) iron absorption ranged from 3.4% to 6.5%. Studies were undertaken to compare non-heme iron absorption from farina meal and evaluated in different laboratories, extensively engaged in human studies of iron absorption. Absorption

Absorption ranged from 5.1 to 10.8%.¹⁰ Similar studies by Hertramf et al.¹¹ showed mean absorption of 6.7% of iron from standard meal. Measured iron absorption from the test meal was 13.0% (Table 1). The values after correction with reference dose and serum ferritin were 14.0% and 16.5% respectively (Table 1) Absorption of test meal was statistically significantly increased ($P<0.01$) compared with standard meal (Table 1). Mean measured reference dose absorption was 49.4% (Table 1) which after adjustment with serum ferritin was 54.2% (Table 1). Absorption of reference dose was statistically significantly increased compared with standard and test meals (Table 1). The measurement of inorganic iron absorption was performed as a reference test to characterize the absorption response of each subject and thereby facilitate comparison of food iron availability performed on different occasions.¹²

In our study correlation between standard and test meal was significant ($P<0.01$, Figure 1). Studies by Cook et al.¹³ showed correlation coefficients for test (dietary) and standard meal absorption to be significant ($r = 0.846$). This shows that the food consumed by most of the people in Pakistan has adequate iron content, the absorption is also adequate but the problem may lie in the feeding habits.

Limitation of the study

The results although from relatively small number of observations, would indicate that iron absorption from diets consumed in developing countries is adequate. However we have identified only one inhibitor of iron absorption (Phytic acid) and one enhancer (ascorbic acid). Similar studies would therefore be needed to confirm the relative effects of phenolic compounds, calcium, vegetable proteins and muscle tissue in adults.

Additional studies are needed on feeding habits of people in Pakistan and iron absorption from whole meals consumed by people in Pakistan. Further investigation of

the technology of iron fortification by various vehicles, including the search for new vehicles and new iron compounds, is required.

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