Non-Heme Iron Availability From Siriraj Hospital Diets

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Abstract : **Absorption of nonheme iron has been studied extensively by the use of extrinsic tag radio-labeled iron added to meals. Nevertheless, this method is unsuitable for screening large numbers of meals. The purpose of this study is to determine and compare iron availability from various kinds of meal in laboratory conditions that mimic the human stomach. Three hundred and seventy two common meals from the Siriraj Hospital kitchen were tested. The results of iron availability tests on the 372 Siriraj meals were classified as having low (5.77 ± 2.42%), intermediate (12.42 ± 1.55%), high (17.7 ± 1.44%) and very high (25.8 ± 3.65%) iron availability. Only meals of high and very high iron availability (22.1 ± 4.99%) provided sufficient amounts of iron for the special requirement of teenagers and women of childbearing age (2.22 to 3.24 mg of Fe per day) while meals of low and intermediate iron availability (8.12 ± 3.84%) provided only 1.02 mg of iron per day. An average amount of estimated iron available from the 372 meals was 14.12 ± 8.2% or 1.77 mg per day. This was slightly inadequate because 2.06 to 2.15 mg were required per day for the high-risk groups. Results of this study indicated that 42.7% of Siriraj meals adequately provided the physiologic requirement of the high-risk segments of the population, but 57.3% of the meals did not. Improvement of food choices to increase dietary iron intake can be accomplished by including meat, fish or poultry and a source of vitamin C in every meal.**

Key words : Non-heme iron availability, radioiron (^{59}Fe), in vitro studies

เรื่องย่อ : **ปริมาณธาตุเหล็กที่ร่างกายนำไปใช้ประโยชน์ได้จากอาหารผู้ป่วยในโรงพยาบาลศิริราช**

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การวัดการดูดซึมธาตุเหล็กในรูปที่ไม่ใช่ฮีมในคนโดยเทคนิคราดิโอไอโซโทปเป็นวิธีที่ได้ผลถูกต้องแม่นยำแต่ไม่เหมาะสมสำหรับการวัดในอาหารประเภทต่าง ๆ ที่มีเป็นจำนวนมาก จึงศึกษาในห้องทดลอง โดยวิธีเลียนแบบระบบย่อยอาหารของคน และใช้เหล็กกัมมันตรังสี (^{59}Fe) เป็นตัวติดตามการแตกตัวของธาตุเหล็กที่ถูกดูดซึมได้ในอาหาร 372 รายการ จากฝ่ายโภชนาการ โรงพยาบาลศิริราช ผลการศึกษาพบว่าความสามารถในการแตกตัวของธาตุเหล็กในอาหารที่ถูกนำไปใช้ประโยชน์ได้ แยกได้เป็น 4 กลุ่ม คือกลุ่มที่ธาตุเหล็กแตกตัวน้อยได้ร้อยละ 5.77 ± 2.42 กลุ่มปานกลางแตกตัวได้ร้อยละ 12.42 ± 1.55 กลุ่มแตกตัวสูงได้ร้อยละ 17.7 ± 1.44 และกลุ่มแตกตัวสูงมากได้ร้อยละ 25.8 ± 3.65 อาหารกลุ่มที่ธาตุเหล็กแตกตัวได้สูงและสูงมากคิดเป็นร้อยละ 42.7 ของจำนวนอาหารทั้งหมด โดยให้ธาตุเหล็กที่ถูกดูดซึมไปใช้ประโยชน์ได้ร้อยละ 22.1 ± 4.99 หรือระหว่าง 2.22 ถึง 3.24 มิลลิกรัมต่อวัน ซึ่งเพียงพอสำหรับวัยรุ่นและหญิงวัยเจริญพันธุ์ แต่อาหารส่วนใหญ่ จำนวน 213 รายการ คิดเป็นร้อยละ 57.3 ของ

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จำนวนอาหารทั้งหมด ให้ธาตุเหล็กที่ถูกดูดซึมได้เพียงร้อยละ 8.12 ± 3.84 หรือ 1.02 มิลลิกรัมต่อวัน ค่าเฉลี่ยจากอาหารทั้งหมดเท่ากับร้อยละ 14.12 ± 8.2 หรือ 1.77 มิลลิกรัมต่อวัน ซึ่งน้อยกว่าค่าความต้องการที่กำหนดโดยองค์การอนามัยโลกสำหรับกลุ่มเสี่ยงคือ 2.06 ถึง 2.15 มิลลิกรัมต่อวัน ผลการสำรวจนี้แสดงว่าร้อยละ 42.7 ของอาหารสำหรับผู้ป่วยเป็นอาหารที่มีโภชนาการของธาตุเหล็กดีมาก ร้อยละ 57.3 มีปริมาณธาตุเหล็กที่ถูกนำไปใช้ประโยชน์ได้ต่ำกว่าค่ากำหนด การคัดเลือกประเภทอาหารหรือปรับส่วนประกอบของอาหารให้มีเนื้อสัตว์หรือวิตามินซีทุกมื้อจะช่วยให้ผู้บริโภคได้รับธาตุเหล็กในอาหารที่ร่างกายสามารถนำไปใช้ประโยชน์ได้เพียงพอกับความต้องการตามเพศและวัย

INTRODUCTION

Iron deficiency is the world's most common nutrient deficiency. Two billion people were reported to suffer from iron deficiency anemia of which about 50% is dietary in origin.[1] Iron deficiency diminishes the body's ability to fight infections, increases the risk of premature delivery, maternal and fetal illness or death, and impairs learning and growth potential in children. The WHO database showed that 50% to 70% of the Southeast Asian pregnant women and school-aged children and 39% of non-pregnant women were anemic.[1-3] Iron deficiency is still the most prevalent nutritional deficiency in Thailand. It has been recently reported that 22% of the population, or 13 million people are anemic and 26 million are iron deficient.[4] To maintain good iron nutrition, a survey of dietary intake from commonly consumed meals must be done so that consumers have more information about their choices of food and how these foods meet the recommended iron intake. Iron availability from meals can be determined simply in the laboratory by the use of an in vitro ^{59}Fe analysis technique.[5-7] This method is the only rapid screening tool available for comparing iron availability from different foods and meals. The primary objective of this investigation was to estimate the amount of iron absorbed from common meals prepared by the Siriraj Hospital kitchen using the in vitro radioisotope technique and to use this technique as a preliminary tool for improving the availability of dietary iron for absorption in the Siriraj meals. It can also be used to optimize the positive and negative interactions between several dietary factors related to iron nutrition.

MATERIAL AND METHOD

Test meals

The Dietary Department of Siriraj Hospital provided 372 regular and special meals for in vitro iron availability analysis.

Total iron content

Test meals were homogenized in deionized water and blended to a creamy consistency in a blender. The total iron content of aliquots of each meal was determined using the wet digestion method[8] and phytate-P was determined using the method of Harland and Oberleas,[9] and iron binding phenolic compounds as tannic acid equivalent was determined using the method of Brune et al.[10] Ascorbic acid was estimated from a food table.[11]

In vitro availability assays

The in vitro radiometric method was used to estimate iron availability after simulated peptic and pancreatic digestion of the food samples.[5-7] Homogenate aliquots of 4 g in 4 ml 0.17 M HCl were first incubated with 1% pepsin in 0.1 M HCl and 1 μCi ^{59}Fe solution at 37°C in a water bath for one hour. The pH of the mixture ranged between 1.3 to 1.5 depending on the buffering capacity of the meal. After incubation, the pH of the mixture was increased to 6.5 by adding acetate buffer (pH 7.5) and 1% pancreatin in 0.1 M $NaHCO_3$ and further incubated for an additional two hours. At the end of this incubation period 1.0 ml chloroform was added to remove suspended lipid droplets. After vortex mixing, samples were centrifuged at 3,000 rpm for 30 minutes.

Triplicate 1.0 ml aliquots of clear supernatant solution were transferred to a clean 6 ml vial and

mixed with 2 ml 0.25 mmol bathophenanthroline in 40% absolute ethanol. Samples were placed on a shaker for 90 minutes and 2.0 ml isoamyl alcohol was added, mixed and centrifuged to separate the alcohol from aqueous phase. One ml aliquots of the alcoholic phase was analyzed for an estimated of radioiron content as a percentage of radioiron presented in the original samples.

Statistical calculations

Statistical calculations were simply made by Microsoft Excel 2000. Group comparisons were done with both the Student t-test and ANOVA by SPSS for Windows.

RESULT

Iron availability from 372 Siriraj meals presented here ranged from 1.44 to 34.98%. The meals were divided into four categories as having low (n=138 or 37.1%), intermediate (n=75 or 20.2%), high (n=71 or 19.1%), and very high availability (n=88 or 23.6%) iron availability. The cut-off values of each category were <10%, 10-15%, 15-20% and >20% as shown in Table 1. The diet category of low and intermediate availability represented 57.3% of the meals and the high and very hish represented 42.7%. The distribution of the iron-availability is shown in Figure 1. The caloric intake from regular and special diets varied between 1,696 and 1,766 kcal (mean 1,730 kcal). An average dietary iron intake was 12.55 mg per day, and the iron nutrient density was 7.4 mg per 1,000 kcal. The content of iron absorption inhibitors ranged from 66 to 111 mg of phytate-P per day, and the content of tannic acid ranged from not detectable to 330 mg per day. The amount of iron enhancer ascorbic acid ranged from not detectable to 138 mg per day.

The mean percentage of iron available for absorption from different types of diets is shown in Table 2. There was a significant difference in estimated iron available between diet categories ($p<0.05$). Iron available from diet category of low availability was significantly lower than that observed in the other categories ($p<0.05$). The iron available from diets of high and very high availability was 2.6 times higher than from the diets of low and intermediate availability ($p<0.001$). The iron available for absorption from diets of low and intermediate group was about 50% lower than the physiologic iron requirement in 20 to 49 years old females. The high and very high group was about 30% higher. As representative of all categories, the mean percentage was about 18% lower. Comparisons between the estimated amounts of available iron for absorption and the physiologic iron requirements according to age and sex of individuals in various populations taken from WHO publications is shown in Figure 2.

Table 3 shows daily iron requirements and iron intake required to reduce risk of developing iron deficiency anemia in different population groups of females. For the diet category of low and intermediate, the total daily amount of iron that the diets must contain to meet the physiological needs of adolescent girls is at least 14.0 mg. For the low category, total amount of iron that the diets must contain is as high as 23 to 37 mg per day to cover the physiologic requirements of all segments of the female population.

DISCUSSION

Various techniques have been developed to predict the availability of iron for absorption. The most useful of these technique is measuring the absorption of nonheme iron from single meals tagged extrinsically with radioactive iron.[12-15] An important limitation of this method is the need to use radioactivity in human subjects. Although the dose of radiation is very small, it must be approved by the Human Ethic Committee and may be more difficult to recruit participants. In this study, a simple in vitro screening method for determining ionisable iron was used to predict the proportion of iron from each meal that was available for absorption. This method is less expensive and requires fewer resources than an in vivo technique, however, it is useful for predicting only a trend, not the magnitude, of the absorptive response in man. The objective of the in vitro method is thus to rank individual meals in order of available iron contents and to predict the relative effects of enhancers and inhibitors of iron absorption.

The quantity of iron in various kinds of food does not necessarily correspond to the amount of iron available for absorption. Dietary iron availability

Table 1. Different iron availability level categorization.

	Level of dietary iron availability			
FAO/WHO 1988[16]	Low 5%	Intermediate 10%	High 15%	
WHO 2001[3]	Very low 5%	Low 10%	Intermediate 12%	High 15%
Siriraj Meals 2003	Low <10% (n = 138)	Intermediate 10-15% (n = 75)	High 15-20% (n = 71)	Very high >20% (n = 88)

Table 2. Percentage of iron available for absorption and estimated absorbed iron (mg/day).

Categories of diets according to iron availability	Iron available for absorption (%)	Estimated absorbed iron (mg/day)*	Percentage below or above requirement**
Low	5.77 ± 2.42	0.724	- 66.3%
Intermediate	12.42 ± 1.55	1.559	- 27.5%
High	17.70 ± 1.44	2.221	+ 3.3%
Very high	25.80 ± 3.65	3.238	+ 50.6%
Low + Intermediate	8.12 ± 3.84	1.019	- 52.6%
High + Very high	22.10 ± 4.99	2.774	+ 29.0%
Total	14.12 ± 8.20	1.771	- 17.6%

*Estimated from mean dietary intake of 372 meals, 12.55 mg/day
**With reference to 2.15 mg required by 20 to 49 years old women[12]

Table 3. Daily iron requirement and dietary iron intakes required in women based on food iron availability.

Daily requirement of (mg/d)	Age group of women (years)				
	13-15	16-19	20-49	50+	Lactating
Absorbed iron	1.76	2.06	2.15	0.96	1.32
Dietary iron from food of					
Low availability (5.77%)	30.5	35.7	37.3	16.6	22.9
Medium availability (12.42%)	14.2	16.3	17.3	7.7	10.6
High availability (17.3%)	9.9	11.6	12.2	5.4	7.5
Very high availability (25.8%)	6.8	8.0	8.3	3.7	5.1
Mean availability (14.2%)	12.4	14.5	15.1	6.8	9.3
Mean iron intake from Siriraj meal = 12.55 mg/d					

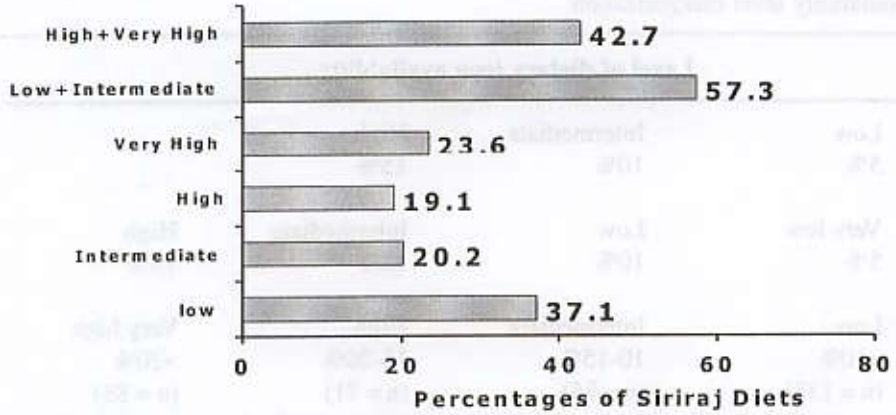


Figure 1. Distribution of food iron availability from Siriraj diets.

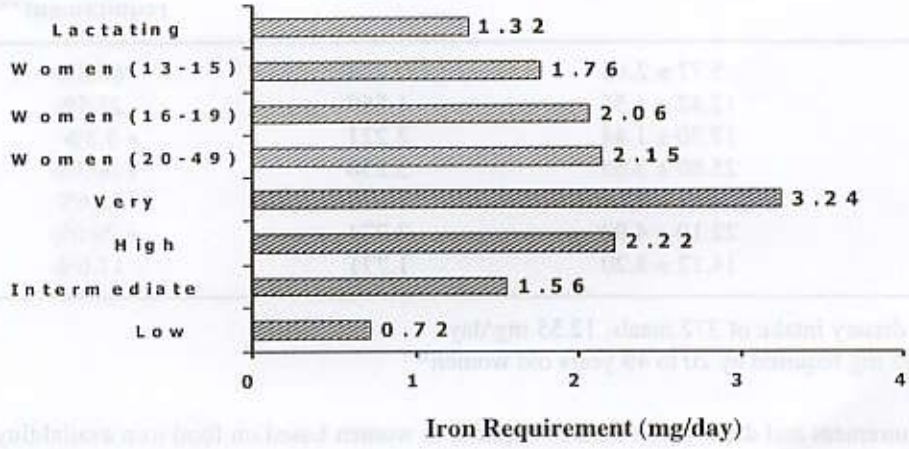


Figure 2. Comparisons between physiologic iron requirements in vulnerable groups and iron available for absorption from meals of different categories of iron availability.

is influenced by the presence of dietary modifiers such as iron absorption inhibitors and enhancers. It has been suggested that the typical diets consumed in various regions of the world could be divided into three categories described as low, intermediate and high bioavailability, with the average absorption from a mixed diets whose ingredients are consumed in different proportions in a non-uniform manner of approximately 5%, 10% and 15% respectively. Siriraj meals were also divided into groups based on FAO/WHO (1988) and WHO (2001) recommendations as shown in table 1.[3,16] It has been identified that low availability meals consist mainly of cereals, legumes and tubes with negligible quantities of meat, fish or ascorbic acid-rich foods and the phytate content is high. An intermediate bioavailability meal consists mainly of cereals but contains some ascorbic acid-rich foods and meat. A high bioavailability diet is a

diversified diet containing generous quantities of meat, poultry, fish and or foods containing a high amount of ascorbic acid. In our surveys, the main ingredients of the meals in each category agreed favorably with those classifications, but it had been observed from the food lists and food compositions that the phytate content was not the main factor lowering the amount of iron available for absorption. Most of the 138 meals in low availability category contained eggs. Iron in eggs is poorly absorbed, unless some ascorbic acid is added.[18] Phosvitin in eggs is the main dietary constituent that inhibits the availability of nonheme iron. A high content of iron binding phenolic compounds, as high as 330 mg tannic acid equivalent, was also observed in several meals of this group. The high and very high diet categories had combinations of 2 to 4 food items. Most of them were special diets which contained more than 100 g of meat and ascorbic acid-rich vegetables and several meals contained seafood. Seafood is an enhancer of iron absorption. Iron levels are not high in seafood, but the iron is easily absorbed.[19-21]

The recommended daily level of iron intake is based on the iron content of foods. A mean concentration of 7.4 mg per 1,000 kcal in the diets can fulfill the needs of all age groups except for adolescents and menstruating women. The iron available for absorption from low and intermediate iron availability of 0.72 and 1.56 mg per day did not meet the physiologic iron requirement in the members of the population who were vulnerable to iron deficiency. The recommended daily intake of iron shown in parentheses in table 3 between 23 to 37 mg per day probably cannot be met unless the diet is fortified with iron.

The results presented here indicated that Siriraj meals in the high and very high availability categories, which accounted for 42.7% of the total meals, could meet physiologic requirements of the risk segments of population but 57.3% meals in the low and intermediate category did not. As the meals presented in this article are consumed by in-patients, it is suggested that meals in the low iron-availability category should be improved to become at least in the intermediate iron-availability category.

CONCLUSION

Iron availability is the proportion of total dietary iron intake that is absorbed and utilized for normal body function. An in vitro iron availability analysis technique based on simulated enzymatic digestion of foods or meals can be used to rank or categorized meals with respect to the effect of a complex interaction of factors on iron availability. Our study demonstrates that this in vitro technique is a useful preliminary tool for predicting the availability of iron for humans. The information available from this study is valuable for improving the selection and preparation of food products with high iron nutrition.

REFERENCES

1. WHO. Nutrition Profile of the WHO South-East Asia Region. WHO Regional Office for South-East Asia, New Delhi, 2000.
2. ACC/SCN, IFPRI. 4th Report on the World Nutrition Situatiom, ACC/SCN, Geneva, 2000.
3. UNICEF/UNU/WHO. Iron deficiency anemia : Assessment, prevention and control. A guide for programme managers. WHO, Geneva, Switzerland, 2001.
4. Winichagoon P. Prevention and control of anemia: Thailand experiences. J Nutr 2002; **132:** S862-66.
5. Rao N, Prabhavathi T. An in vitro methods for predicting the bioavailability of iron from foods. Am J Clin Nutr 1978; **31:**169-75.
6. Miller DD, Schricker BR, Rasmussen RR, Van Campen D. An in vitro method for estimation of iron availability from meals. Am J Clin Nutr 1981; **34:** 2248-56.
7. Tuntawiroon M, Sritongkul N, Pleehachinda R, Suwanik R. Estimation of available dietary iron, in vivo and in vitro comparisons. Thai Nuc Med Newsletter 1998; **4:** 46-51.
8. Official Methods of Analysis of the Association of Official Agricultural Chemists (AOAC.) 10th ed. Washington DC: Association of Official Agricultural Chemists, 1965: 192.
9. Harland B, Oberleas D. Anion-exchange method for determination of phytate in foods: Collaborative study. J Assoc Offic Anal Chem 1986; **69:** 667-70.
10. Brune M, Hallberg L, Skanberg AB. Determination of iron binding phenolic compounds. J Food Science 1991; **56:** 128-31.
11. Nutrition Division, Ministry of Public Health. Nutritive values of Thai Foods, 1992.

12. Cook JD, Layrisse M, Martinez-Torres C, Walker R, Monsen E, Finch CA. Food iron absorption measured by an extrinsic tag. J Clin Invest 1972; **51:** 805-15.
13. Hallberg L, Bjorn-Rassmussen E. Determination of iron absorption from whole diet. A new two-pool model using two radioiron isotopes given as heme and nonheme iron. Scand J Haematol 1972; **9:** 193-97.
14. Tuntawiroon M, Sritongkul N, Brune M, Rossander-Hulten L, et al. Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in man. Am J Clin Nutr 1991; **53:** 554-57.
15. Tuntawiroon M, Sritongkul N, Rossander-Hulten L, Pleehachinda R, et al. Rice and iron absorption in man. Eur J Clin Nutr 1990; **44:** 489-97.
16. FAO/WHO (1988). Requirements of vitamin A, iron, folate, and B_{12}. Report of a joint FAO/WHO consultation. Food and Nutrition Series No. 23. Rome.
17. Rossander L, Hallberg L, Bjorn-Rasmussen. Absorption of iron from breakfast meals. Am J Clin Nutr 1979; **32:** 2484-89.
18. Monsen ER, Cook JD. Food iron absorption in human subjects. IV. The effect of calcium and phosphate salt on the absorption of nonheme iron. Am J Clin Nutr 1976; **29:** 1142-48.
19. Cook JD, Monsen ER. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am J Clin Nutr 1976; **29:** 859-67.
20. Hallberg L. Bioavailability of dietary iron in man. Ann Rev Nutr 1981; **1:** 123-47.
21. Taylor PG, Martinez-Torres C, Romano EL, Layrisse M. The effect of cysteine-containing peptides releases during meat digestion on iron absorption in human. Am J Clin Nutr 1986; **43:** 68-71.