

Estimation of Available Dietary Iron, In Vitro and In Vivo Comparisons

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The *in vitro* method presented in this study is capable of distinguishing between iron availabilities from varieties of composited meals. It provides a simple and reproducible method for predicting iron availability from a complex mixture of foods. This method also allows rapid and inexpensive screening for known enhancers and inhibitors of food iron absorption, and correlates closely with percentage absorption measured in human volunteers.

Keywords: *iron absorption, iron availability, in vitro ionisability*

INTRODUCTION

The amount of nonheme iron absorbed from the diet is determined not only by its content of iron but also by the balance between different dietary factors enhancing or inhibiting iron absorption. Several dietary factors have been identified to influence iron bioavailability. Addition of ascorbic acid, meat and fish significantly increased absorption of nonheme iron whereas several others inhibit iron absorption such as phytate and iron binding phenolic compounds⁽¹⁻³⁾.

Absorption of nonheme iron has been studied extensively by the use of extrinsic radioiron labelled added to meals^(4,5). Nevertheless, these *in vivo* methods are time consuming, expensive and unsuited for screening large numbers of meals. A variety of *in vitro* methods have been designed⁽⁶⁻⁹⁾ by simply incubating a food with pepsin-HCl followed by pancreatin digestion to simulate human digestive function. The present study was undertaken to

compare *in vitro* radiometric method, based on the same approaches, and the human extrinsic radioactive tag methods in determining dietary availability for iron from Thai meals with or without the effect of absorption inhibitors and promoters.

MATERIALS AND METHODS

Test meals

Iron absorption and *in vitro* ionisability for determining food iron availability were measured in a parallel manner from 24 commonly consumed rice-based Thai meals compiled from 9 series of studies during the period from 1985 to 1993. All meals were prepared as is normally done in the laboratory kitchen. The main components of all meals were rice-vegetable-meat (either pork or poultry), except meal 21, 22, 23, and 24, they were soy protein vegetarian meals. Samples for *in vitro* measurement were blended into a homogeneous slurry. Aliquots of each meal were taken for total iron content⁽¹⁰⁾, total

phosphorus and phytic acid phosphorus⁽¹¹⁾, and iron binding phenolic compounds⁽¹²⁾. Ascorbic acid was estimated from food table⁽¹³⁾. Chemical composition of meals is shown in Table 1. Iron binding phenolic compounds were undetectable in any test meal.

Subjects

A total of 129 male subjects participated in 24 studies of 9 series were healthy volunteers, aged 20 to 49 years. They were given oral and written information about the aims and procedures of the study. The project and protocol were approved by the Human Subjects Committee of Siriraj Hospital Medical School.

Iron absorption measurements

Iron absorption was measured from 2 or 3 separate test meals in each subjects, a total of 24 test meals in 129 subjects, by the use of sequential ⁵⁵Fe and ⁵⁹Fe labelled^(4,5). All test meals were given at 12.00 noon after an overnight fast and a standard breakfast served at 7.30 a.m.^(14,15) After the meals no foods or drinks was allowed for 3 hours. Each test meal was labelled with 46.3kBq ⁵⁹Fe or 55.5 kBq ⁵⁵Fe, carrier free, high specific activity in 0.1 M HCl/litre. A blood sample was drawn 2 weeks after serving the first and second test meals on alternate days and a final blood samples was obtained 2 weeks after the third meals to measure the increase in red cell radioactivity. Measurements of blood radioactivity were performed on duplicate 10 ml specimens of whole blood by the method modified by Eakins and Brown⁽¹⁶⁾. Percentage absorption was calculated on the basis of blood volume estimated from weight⁽¹⁷⁾.

In vitro measurements

Our in vitro method for determining relative iron availability resembles several of the previously published in vitro methods^(6-9,18) but with some minor modifications to suit our laboratory facilities. After test meal was homogenized to a creamy consistency, aliquots of 4 g in 4 ml 0.17 M HCl were first incubated with 1% pepsin in 0.1 M HCl and 1 μ Ci ⁵⁹Fe solution at 37°C for 1 hour. The pH of the mixture was range 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 Na₂HCO₃ and the incubation was continued for an additional 2 hours. At the end of this incubation 1 ml chloroform was added to remove suspended liquid droplets. After vortex mixing, they were centrifuged at 3000 rpm for 30 minutes.

Triplicate 1 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 ml isoamyl alcohol was added, mixed and centrifuged to separate the alcohol from aqueous phase. A 1 ml aliquots of the alcoholic phase was taken for estimation of radioiron content as a percentage of radioiron presented in the original samples.

Statistical analysis

All data were collected during the period from 1985 to 1993, the calculations and statistical analyses were made by using SPSS program for Windows. The in vitro data were compare with the in vivo data by correlation analysis.

RESULTS AND DISCUSSION

The results of iron absorption along with iron ionisability carried out on 24 different test meals were collected during the period from 1985 to 1993 (Table 2). The recorded results of in vitro measurements show a small degree of variability, between 2 to 2.5 % in 15 % of the test samples and the rest 85 % were less than 2 %. Correlation analysis indicate significant agreement between in vitro and human in vivo methods. The correlation coefficient being 0.931 ($p < 0.001$). A straight line relationship can be expressed by a prediction equation $Y = 0.3145 + 0.4312X$ where Y

is the percent iron absorption in adult male volunteers and X is the percent ionisable iron (Figure 1).

The absorption of nonheme iron is markedly influenced by the nature of the meal. In this studies, most of the in vitro results showed the same significant effect in the presence of either enhancing or inhibiting substances. The ionisable iron was shown to decrease considerably when varying amount of phytic acid was included in the meal (Study II, meal 2 to 5) and increase considerably when the diet included ascorbic acid (Study III, meal 6 to 9 and Study IX, meal 22 to 24). These indicate

Table 1 Composition of meals

Study Meal	Test meal main ingredients	Energy (kcal)	mg per meal			
			Fe	P	Phytate	AA*
I 1	Rice-vegetable-fish	475	3.04	144	30	10
II 2	Rice-vegetable-pork + 35mgPhy	680	3.20	184	35	10
II 3	Rice-vegetable-pork + 60mgPhy	685	3.12	201	60	10
II 4	Rice-vegetable-pork + 115mgPhy	690	4.00	250	115	10
II 5	Rice-vegetable-pork + 175mgPhy	695	3.62	294	175	10
III 6	Rice-vegetable-pork + 60mgPhy + AA25	685	3.20	184	60	35
III 7	Rice-vegetable-pork + 60mgPhy + AA50	685	3.20	184	60	60
III 8	Rice-vegetable-pork + 115mgPhy + AA25	690	4.00	250	115	35
III 9	Rice-vegetable-pork + 115mgPhy + AA50	690	4.00	250	115	60
IV 10	Rice-chicken curry	568	3.00	144	39	0
IV 11	Rice-chicken-curry + AA50	568	3.00	165	35	50
V 12	Rice-vegetable-pork curry	739	3.84	154	36	0
V 13	Rice-vegetable-pork with hot chili	570	2.73	167	41	0
V 14	Rice-sweet and sour soup	480	2.17	70	22	0
VI 15	Rice-chicken curry	560	2.30	144	31	0
VI 16	Rice-chicken curry + raw cucumber	570	2.75	184	31	3
VI 17	Rice-chicken curry + cook cucumber	570	2.45	184	31	3
VII 18	Rice-chicken curry	560	2.23	144	31	0
VII 19	Rice-chicken curry + AA5	575	2.43	156	31	5
VII 20	Rice-chicken curry + AA20	575	2.38	156	31	20
VIII 21	Rice-vegetable-mushroom soup	547	2.44	144	46	0
IX 22	Rice-soybean with chili	583	3.01	143	91	0
IX 23	Rice-soybean with chili + AA100	583	3.01	143	91	100
IX 24	Rice-soybean with chili + AA200	583	3.01	143	91	200

* Total amount of ascorbic acid per meal

Table 2 Percent ionisable iron for estimation of food iron availability and iron absorption in different test meals

Study	Meal	n	Test meal main ingredients	Ionisable iron %	Iron absorption %
I	1	12	Rice-vegetable-fish	24.49 ± 1.36*	10.46 ± 1.36*
II	2	11	Rice-vegetable-pork + 35 mgPhy	42.60 ± 0.23	22.10 ± 3.34
II	3	8	Rice-vegetable-pork + 60 mgPhy	29.20 ± 0.29	13.37 ± 2.39
II	4	10	Rice-vegetable-pork + 115 mgPhy	20.70 ± 1.82	10.56 ± 2.12
II	5	11	Rice-vegetable-pork + 175 mgPhy	17.50 ± 2.25	7.51 ± 1.46
III	6	10	Rice-vegetable-pork + 60 mgPhy + AA25	27.10 ± 1.04	14.06 ± 2.31
III	7	10	Rice-vegetable-pork + 60 mgPhy + AA50	45.50 ± 0.64	15.09 ± 1.92
III	8	9	Rice-vegetable-pork + 115 mgPhy + AA25	18.10 ± 0.29	8.77 ± 1.31
III	9	9	Rice-vegetable-pork + 115 mgPhy + AA50	35.20 ± 2.22	13.67 ± 1.70
IV	10	12	Rice-chicken curry	39.70 ± 2.51	19.60 ± 2.48
IV	11	12	Rice-chicken-curry + AA50	69.50 ± 1.33	33.00 ± 4.52
V	12	11	Rice-vegetable-pork curry	14.56 ± 0.29	6.24 ± 2.32
V	13	11	Rice-vegetable-pork with hot chili	37.25 ± 0.64	11.40 ± 4.19
V	14	11	Rice-sweet and sour soup	62.07 ± 0.94	22.90 ± 4.88
VI	15	13	Rice-chicken curry	30.60 ± 0.91	14.10 ± 2.10
VI	16	13	Rice-chicken curry + raw cucumber	31.20 ± 1.67	13.90 ± 2.20
VI	17	13	Rice-chicken curry + cook cucumber	39.10 ± 2.20	19.60 ± 2.80
VII	18	11	Rice-chicken curry	32.30 ± 1.25	14.30 ± 2.70
VII	19	11	Rice-chicken curry + AA5	31.2 ± 1.67	15.40 ± 2.90
VII	20	11	Rice-chicken curry + AA20	36.30 ± 0.78	20.20 ± 2.80
VIII	21	13	Rice-vegetable-mushroom soup	9.40 ± 1.50	4.10 ± 0.53
IX	22	11	Rice-soybean with chili	12.30 ± 1.57	3.50 ± 0.66
IX	23	11	Rice-soybean with chili + AA100	22.70 ± 0.24	9.20 ± 1.90
IX	24	11	Rice-soybean with chili + AA200	32.00 ± 1.03	12.50 ± 1.84

* Mean ± SEM

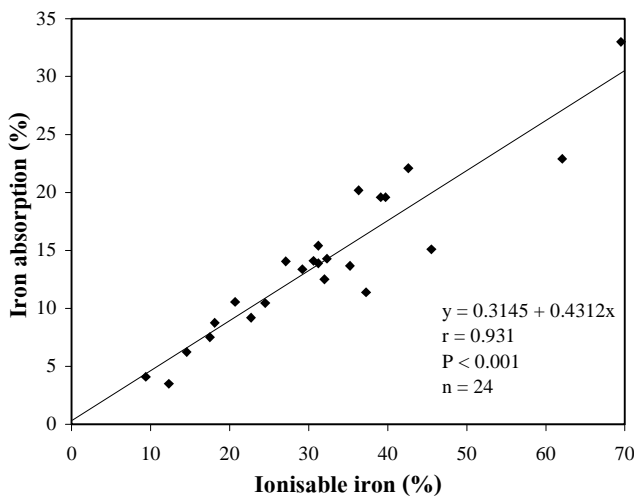


Fig. 1 Regression line fitted to scatter diagram of percent in vivo iron absorption on percent in vitro ionisable iron.

that the radiometric in vitro technique is reproducible and responsive to factors known to affect food iron availability. In most of the examples presented in this paper, in vitro studies have yielded results that reliably predict nonheme iron availability in man. It is simple and applicable to meals of varying nutrient composition, and, as the quantity of available iron in a meal can be accurately predicted, nutrition may be improved by pre-estimating the adequacy of diets with respect to iron.

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