Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in men[1-3]

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ABSTRACT Nonheme-iron absorption from a typical Southeast Asian meal was studied to examine the effect of a common vegetable, Yod Kratin, which contains a considerable amount of iron-binding phenolic groups. Yod Kratin (leaves of the lead tree) is a very popular vegetable in Thailand. It is consumed at least once a week year round, sometimes every day, together with the main meal. With a common portion size of the vegetable (20 g), iron absorption was reduced by almost 90%. As little as 5 g inhibited iron absorption by 75%. Addition of ascorbic acid partly counteracted inhibition. Adding 100 mg ascorbic acid reduced inhibition of iron absorption from 5 g Yod Kratin by half and the inhibition from 10 g Yod Kratin by a quarter. The study illustrates the marked effect of iron-binding phenolic compounds on iron nutrition and, thus, the importance of acquiring knowledge of the content of such compounds in different foods. *Am J Clin Nutr* 1991;53:554–7.

KEY WORDS Iron absorption, men, iron-binding phenolic compounds, ascorbic acid, vegetable

Introduction

The main cause of iron deficiency is nutritional, ie, the diet cannot cover physiological requirements. 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. Knowledge about such factors has grown much over the last 15–20 y with the introduction of the method of extrinsic labeling of dietary iron (1, 2). With extrinsic labeling it became possible to measure iron absorption from single meals and to identify and quantify the effect of various factors in the diet on the absorption of iron. The main enhancing factors are ascorbic acid and meat, fish, and poultry. One main inhibitor of iron absorption is the phytate present in cereals, certain vegetables, roots, nuts, etc. Another main inhibitor is a group of compounds, usually tannins or polyphenols, that are present in tea, coffee, certain spices, fruits, and vegetables (3).

Thousands of phenolic compounds are described in the chemical literature. These compounds have one or more aromatic rings, bearing hydoxyl groups in different patterns. They may vary in size from small simple molecules to huge polymerized complexes. In the plant kingdom these compounds are probably a normal defense system. The complexity and heterogeneity of phenolic compounds are reasons why there is no specific method available for their determination. The methods used measure a single property, such as ability to react with proteins (4–6).

Knowledge about the inhibiting effect of various phenolic compounds on iron absorption is limited. It has been known for a long time that some of these compounds may form colored complexes with iron. A series of studies on the properties of such complexes resulted in the development of a specific method for quantifying the amount of catechol and galloyl groups present in phenolic compounds (7). The galloyl groups turned out to be mainly responsible for the binding of iron such that its absorption was inhibited (8).

Thus, a new field of research was opened up. Systematic studies on the content of iron-binding groups in various foods such as vegetables, fruits, cereals, and spices were started to find the reason for the often inexplicably high prevalence of iron deficiency in some populations in developing countries and to explore new and more effective ways to combat iron deficiency through food education.

The present studies were undertaken to study, under realistic conditions, the relationship between the content of such iron-binding phenolic compounds in a meal and the extent of inhibition of iron absorption. A very common and popular vegetable in Thailand, Yod Kratin (leaves of the lead tree, *Leucaena glauca*), was chosen for the present studies. The amounts of vegetable served were well within the usual range for its practical use. Ascorbic acid markedly enhances the absorption of dietary iron (9) and strongly counteracts the inhibitory effect of phytate on iron absorption (10). Thus, another purpose of the present studies was to examine the possibility of counteracting the inhibition of phenolic compounds on iron absorption by adding ascorbic acid in amounts that may be nutritionally realistic.

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Subjects and methods

Experimental design

A common meal in Thailand composed of rice, fried fish (Pla Tu), a curry (Nam Prik Kapi) and different amounts of a vegetable (Yod Kratin) was served to healthy male volunteers. Each subject was served three meals (A, B, and C) and one reference dose of iron (R). One group of subjects was served meals 1, 3, and 5 and another group meals 2, 4, and 6. All volunteers were instructed to fast from midnight until 0730 when a standard breakfast consisting of boiled rice and salted cabbage was served. The test meals were served at 1200. After the meals were served, no food or drink was allowed for 3 h.

Four iron-absorption measurements were made in each subject and these were made as two consecutive pairs, A-B and C-R, respectively. The A and B meals were served on alternate days and were labeled with two different radioiron isotopes, ^{55}Fe and ^{59}Fe. A fasting blood sample was drawn 2 wk later. The C meal and the reference dose were then given on alternate days and labeled with the two radioiron isotopes. A final blood sample was drawn 2 wk later to measure the increased radioactivity in circulating red cells. All procedures and methods of calculations were described previously (11).

The studies on the effect of ascorbic acid were made in a similar way. In one group of subjects, the same meals as above were served with 5 g Yod Kratin (wet weight) containing 0, 50, or 100 mg ascorbic acid. Four iron-absorption measurements were made in each subject, including a reference-dose absorption measurement. As above, they were made as two pairs of studies. In another group of subjects, the same comparisons were made but the meals were served with 10 g Yod Kratin.

Subjects

In total, 83 male volunteers participated in the studies. They considered themselves quite healthy. They were all workers or employees at the Siriraj Hospital, Bangkok, and aged 20–40 y. The project and protocol were approved by the Human Subjects Committee of Siriraj Hospital Medical School, Mahidol University, Bangkok.

Methods

A solution of 10 mL of 0.01 mol HCl/L containing 3 mg Fe as $FeSO_4$ and 30 mg ascorbic acid labeled with ^{59}Fe was used as a reference in all studies. The 10-mL vials containing the Fe solution were rinsed twice with water and the washings were consumed. Each subject received the reference dose in the morning after an overnight fast. No food or drink was allowed for 3 h after the reference dose. Each subject received a dose of 46 kBq ^{59}Fe.

The rice-vegetable-fish-curry meals had the following composition: each meal contained 50 g fried fish (Pla Tu), 100 g polished rice cooked in 150 mL deionized water, 55 g Nam Prik Kapi [composed of 5.5 g garlic, 2.5 g small red chilli, 15 g shrimp paste, 10 g sugar, 3.5 g fish sauce (a salt solution), and 18.5 mL lemon juice]. The Nam Prik curry contains only negligible amounts of tannins. Different amounts of the vegetable Yod Kratin, which was consumed raw, were served with the meals. The amount of Yod Kratin varied from 0 to 20 g.

In all these meals, the radioiron was mixed into the cooked rice. Each meal was labeled with 46.3 kBq ^{59}Fe or 55.5 kBq ^{55}Fe. Carrier-free, high-specific-activity radioiron in 0.1 mol HCl/L was used.

Samples of foods were freeze-dried and ground to a powder in a porcelain mortar. Weighed amounts were analyzed for total iron (11), phytate phosphorus (12), and iron-binding phenolic compounds (7).

One gram Yod Kratin contained 29.2 tannic acid equivalents. The variation between samples of Yod Kratin was small. In 24 samples the SEM was only ±0.73 mg/g wet wt. No catechin equivalents or phytate were found. The basal meal contained only traces of iron-binding phenolic compounds (in the Nam Prik curry). It contained 38 mg of phytate phosphorus.

Statistical analyses

The ratio (A:R) of absorption of nonheme iron from a meal (A) and from reference doses (R) is an expression of the bioavailability of nonheme iron in a meal. The distribution of these ratio values is normal, and the mean and SEMs of the ratio values are calculated in the usual way. The mean values of these ratios and their SEMs were multiplied by 40 to obtain the percentage absorption of iron that corresponds to a 40% reference-dose absorption ($A_{40\%}$). Absorption values adjusted to a 40% absorption from reference doses were chosen because they correspond to the absorption expected in subjects who are borderline iron deficient (13). Because results are expressed as geometric means by some researchers in the iron field, these means are also included in the tables to facilitate comparisons. Standard statistical analysis-of-variance (ANOVA) methods were used to analyze the data (*Statview II,* Abacus Concepts, Inc, Berkeley, CA). Groups were compared by using the methods of Fisher's protected least-significant difference and Scheffé's S procedure.

Results

The results are presented in **Tables 1 and 2** and in **Figure 1.** It is evident that with increasing amounts of Yod Kratin there is a successive decrease in the absorption of iron. The maximal mean decrease in iron absorption through the addition of Yod Kratin was 87%. There was a 50% decrease in iron absorption after the administration of as little as 3 g Yod Kratin, corresponding to 87.6 mg tannin equivalents. This decrease was highly statistically significant ($P < 0.001$). Administration of 5 g Yod Kratin further reduced absorption ($P < 0.01$). Iron absorption

TABLE 1
Effect of different amounts of Yod Kratin on iron absorption from a composite meal

Group	Yod Kratin content	Tannic acid equivalent	Iron content	Iron absorption (A_{40})*
	g/meal	*mg/meal*	*mg*	*%*
1 (n = 10)	0	0	5.36	12.8 ± 1.83 (11.7 ± 1.15)
2 (n = 11)	3	87.6	5.03	6.4 ± 1.35 (5.0 ± 1.25)
3 (n = 10)	5	146	5.20	3.5 ± 0.46 (2.8 ± 1.27)
4 (n = 11)	10	292	6.05	2.4 ± 0.49 (2.0 ± 1.22)
5 (n = 10)	15	438	5.52	2.0 ± 0.46 (1.6 ± 1.23)
6 (n = 11)	20	584	5.80	1.7 ± 0.47 (1.3 ± 1.27)

* $\bar{x}$ ± SEM; geometric $\bar{x}$ ± SEM given in parentheses.

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TABLE 2
Effect on iron absorption of adding different amounts of ascorbic acid (AA) to two meals with different amounts of Yod Kratin

Yod Kratin content	Iron absorption (A_{40})*		
	0 mg AA	50 mg AA	100 mg AA
g/meal			
5 (n = 9)	3.4 ± 0.46	5.4 ± 2.05	7.6 ± 1.86†
	(2.8 ± 1.38)	(4.5 ± 1.31)	(6.4 ± 1.27)
10 (n = 11)	2.8 ± 0.84	4.2 ± 1.28†	4.9 ± 1.42‡
	(1.6 ± 1.40)	(2.6 ± 1.37)	(3.2 ± 1.47)

* $\bar{x}$ ± SEM; geometric $\bar{x}$ ± SEM given in parentheses.
†‡ Significantly different from 0 mg; †$P < 0.05$, ‡$P < 0.01$.

decreased more with additional increases of Yod Kratin. ANOVA, however, showed that this further decrease was not statistically significant.

At two levels of Yod Kratin administration, 5 and 10 g, corresponding to 146 and 292 mg tannic acid equivalents, the counteractive effects of adding 50 or 100 mg ascorbic acid were examined. The administration of ascorbic acid consistently increased the absorption of iron. For both levels of Yod Kratin, 50 mg ascorbic acid increased the absorption by 50% and 100 mg doubled the absorption (Table 2). At the lower level of Yod Kratin (5 g) studied, the effect of ascorbic acid only reached statistical significance when 100 mg was given. With 10 g Yod Kratin, the effect of both 50 and 100 mg ascorbic acid was statistically significant. In spite of the rather marked effect of ascorbic acid, however, it was not possible to fully overcome the inhibition of iron absorption by Yod Kratin even with 100 mg ascorbic acid. By and large, 100 mg of ascorbic acid reduced inhibition by half and 50 mg reduced it by only 25%.

Discussion

In the rural areas throughout Thailand, Yod Kratin is the most commonly eaten vegetable. It grows almost everywhere, often as a fence around the garden, and usually is eaten with the main meal in the evening. It is consumed almost every day and at least once a week in all seasons. The usual portion size is 20–50 g. The present meal studied is the most common combination. Sometimes cucumber and eggplant are also included in these meals. The bioavailability of iron in the basal meals not containing Yod Kratin, 12.8%, must be considered to be good.

In the present study the portion size of Yod Kratin was only 3–20 g. The reason for this was the high content of galloyl groups and the expected marked inhibition of the absorption of iron. The inhibiting effect of Yod Kratin on the absorption of iron was very pronounced. Iron absorption was reduced by 90% after 20 g of the vegetable was eaten. Our findings illustrate the importance of iron-binding phenolic compounds for iron nutrition. Frequent and regular consumption of Yod Kratin with the main

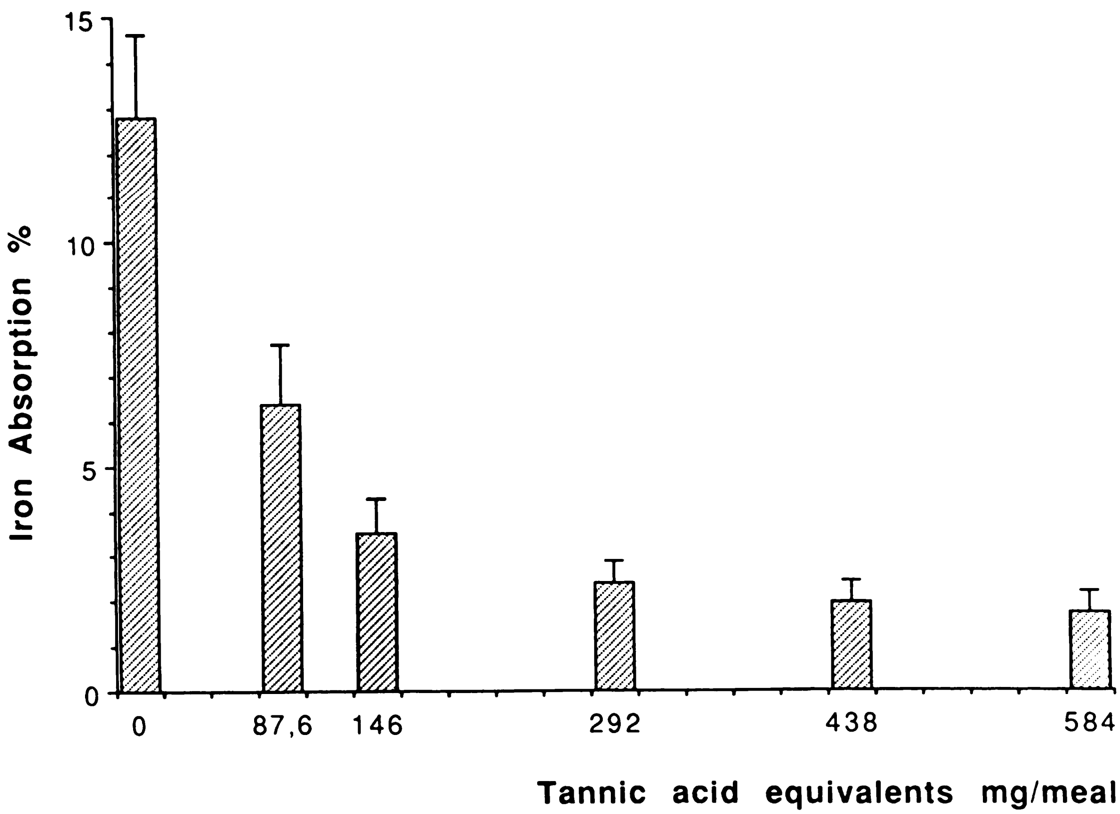

FIG 1. Iron absorption from meals containing rice, fish, and various amounts (0–20 g) of a vegetable (Yod Kratin) containing different amounts of iron-binding phenolic compounds expressed as tannic acid equivalents (mg). $\bar{x}$ ± SEM.

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meal could easily reduce the total dietary iron absorption by half.

Yod Kratin is quite popular because it both tastes good and is inexpensive. The feasibility of exchanging it for another vegetable must be carefully explored. A food that counteracts inhibition of iron absorption, eg, an ascorbic-acid-rich vegetable, could be added to a meal containing Yod Kratin. However, the present findings of a rather limited antagonistic effect of ascorbic acid on inhibition by large amounts of iron-binding phenolic compounds are not too encouraging. Ascorbic acid has a dramatic counteractive effect on the inhibition of iron absorption by phytate. It is reasonable to assume that the observed, rather modest counteracting effect of ascorbic acid on the inhibition of iron absorption by Yod Kratin is due to the huge amounts of galloyl groups present in this vegetable. Our results clearly show that important tasks exist in identifying foods with a high content of iron-binding phenolic compounds, in searching for alternative foods, and in finding more effective ways to neutralize the inhibiting effect of these compounds.

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