

## Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour<sup>1-3</sup>

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**ABSTRACT** The bioavailability in man of commercially available elemental iron powders is unknown despite their extensive use for fortification of flour. Carbonyl iron, which is widely used in Europe, is considered as one of the best reduced iron powders based on studies both in vitro and in animals. In this study, a <sup>55</sup>Fe labeled carbonyl iron was prepared by neutron irradiation and used to fortify wheat flour. The native iron of the wheat was extrinsically labeled by <sup>59</sup>FeCl<sub>3</sub>. Doubly labeled wheat rolls were served with different meals. The ratio of absorbed <sup>55</sup>Fe/<sup>59</sup>Fe is a direct measure of the fraction of carbonyl iron that joins the nonheme iron pool and is made potentially available for absorption. This relative bioavailability of carbonyl iron was unexpectedly low and varied from 20 to 5% when the iron fortified wheat rolls were served with different meals. The baking process did not change the relative bioavailability nor the addition of ascorbic acid. The low and variable bioavailability of carbonyl iron in man, makes it necessary to reconsider the rationale of using elemental iron powders for the fortification of foods for human consumption. *Am J Clin Nutr* 1986;43: 59-67.

**KEY WORDS** Iron fortification, carbonyl iron, man, bioavailability, meals, radioiron methodology

Fortification of the diet with certain nutrients is considered as an important public health measure to prevent deficiency disorders. In industrialized countries these deficiencies may be caused by an increased use of refined foods such as wheat flour of low extraction, or a lower intake of energy due to the lower energy expenditure of recent generations which in turn is often accompanied by a critically low intake of certain essential nutrients such as iron. This is especially valid for children, teenagers, and women of childbearing age. The rationale of improving iron nutrition in these groups has received substantial support in recent years from studies showing the negative effects of iron deficiency, not only as it relates to anemia, but also to a lack of iron for iron dependent enzymes which may be rate limiting for physical work performance, certain learning processes, body temperature control, etc. For a review see ref 1.

Iron fortification is technically difficult. The challenge is to find a vehicle that reaches the target groups in an optimal way and to find an iron compound that is both effective and compatible with the vehicle. In western countries flour and infant cereals are the most common vehicle for iron fortification. Various

forms of elemental iron powders are the most commonly used iron preparations for this purpose as they do not cause the severe technical problems typical with easily soluble iron salts such as ferrous sulfate. These iron salts are easily available for absorption but cause discoloration by reacting with various items in the food or by catalyzing oxidative reactions in the food causing unwanted flavors and odors. The elemental iron powders (reduced iron) are chemically more inert and induce fewer technical problems. However, a major problem with the iron powders is that their bioavailability in man is unknown, despite its very wide use in commercially available products. For a review see ref 2.

There are three methods for manufacturing elemental iron: 1) Electrolytic deposition, 2)

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reduction of iron oxide by hydrogen or carbon monoxide, and 3) production by the carbonyl process. This involves two steps, first the production of iron pentacarbonyl and then its decomposition and further reduction by hydrogen. This forms very uniform and very small iron particles. Over the years the properties of the elemental iron powders have been carefully studied with respect to their bioavailability in animals. Usually the hemoglobin repletion in iron deficient rats has been used and the effect of various iron preparations have been compared with the effect observed when ferrous sulfate is given. The iron powders have also been characterized with respect to particle size distribution, solubility in dilute hydrochloric acid, and reactive surface area. The efficacy of some of the early elemental iron powders has been seriously questioned, especially the bioavailability of those containing a large proportion of coarse particles. The earlier studies have recently been reviewed (3, 4).

Elemental iron powder produced by the carbonyl process (carbonyl iron) appear to be one of the best among the commercially available products, as the iron particles are very small and uniform and are readily soluble in dilute hydrochloric acid (5, 6). Moreover, hemoglobin repletion studies in rats have shown that carbonyl iron and ferrous sulfate are equally effective when baked into bread and that carbonyl iron is better in the repletion test than electrolytic iron or iron reduced by hydrogen or carbon monoxide (7).

The good bioavailability of carbonyl iron in animal studies and its good physicochemical properties had led to a widespread use of this preparation in iron fortification of flour. In some countries a great proportion of the total iron intake comes from iron fortification—in Sweden as much as 42% (8).

For these reasons it seems imperative to study the bioavailability of carbonyl iron in man and to do this under realistic dietary conditions.

This was achieved in the present study by making a commonly used carbonyl iron preparation radioactive by neutron irradiation which could be done without changing its other physicochemical properties in any measurable way.

The present paper reports studies in man on the bioavailability of carbonyl iron mixed

with flour in amounts to correspond to commonly used levels of iron fortification (2.5–7.5 mg iron/100 g flour). Wheat rolls baked with this flour were served together with different types of meals. In some of the meals the effect of ascorbic acid on the relative bioavailability of the carbonyl iron was also studied.

### Material and methods

The investigation was comprised of 15 studies and a total of 140 subjects. Hematological and other data describing these subjects are given in Table 1.

Studies 1–4 measured the relative bioavailability of carbonyl iron in different meals. To each of these meals 1 mg of carbonyl Fe was added, see Table 2. Studies 5–7 examined the question of whether the relative bioavailability of carbonyl iron was dose-related. In studies 8–11 the effect of adding 25 or 50 mg ascorbic acid on the relative bioavailability of carbonyl iron was measured. In study 12 the effect of fermentation and baking on the relative bioavailability of carbonyl iron was investigated. The breakfast meal was composed of porridge made from wheat flour containing 1 mg of carbonyl iron served with coffee and the same amount of jam as in the other breakfast studies. Study 13 compared the absorption of 1 mg carbonyl iron ( $^{55}\text{Fe}$ ) with 1 mg of ferrous sulfate iron ( $^{59}\text{Fe}$ ) from two identical breakfast meals alternately served on four consecutive days. In studies 14 and 15, for a further basis on comparison, the absorption of nonheme iron was measured from unfortified meals.

The study was approved by the Ethical Committee of the University of Göteborg. A consent was obtained from each volunteer after a detailed oral and written explanation about the investigation.

### Experimental design

A commercial product of carbonyl iron (BASF-type CP 2161) was neutron irradiated to induce the formation of  $^{55}\text{Fe}$ . This product was left standing for a considerable time to reduce the content of contaminating radioactive isotopes of other elements formed during the irradiation process (see below).

The  $^{55}\text{Fe}$  labeled carbonyl was used to fortify wheat flour. The native iron in the wheat flour was labeled by adding a trace amount of  $^{59}\text{Fe}$  labeled ferric chloride. Wheat rolls were baked from this doubly labeled flour except in one experiment when a porridge was made of the flour to study the effect of baking on the bioavailability of the carbonyl iron.

The wheat rolls were served with different meals in the morning to subjects who had fasted overnight. No food or drink was allowed for the first 3 h after serving the meals. The same doubly radioiron labeled meals were served twice on two consecutive mornings. Two weeks after the last serving, a blood sample was drawn to measure  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  and a whole body counting of  $^{59}\text{Fe}$  was also made. A reference solution of  $^{59}\text{Fe}$  labeled ferrous ascorbate (3 mg Fe) was then given on two consecutive mornings after an overnight fast and 2 wk later a new whole body counting was done to measure the retention of the iron

TABLE I  
Characteristics of subjects (mean values  $\pm$  SEM)

Series	No and sex of subjects*	Age	Height	Weight	Hb	Hematocrit
		yr	cm	kg	g/l	%
1	2 M (2) 8 F (1)	37	176.5 $\pm$ 3.5	67.0 $\pm$ 7.0		
		24	167.8 $\pm$ 1.6	58.0 $\pm$ 3.9	141 $\pm$ 9	42.0 $\pm$ 2.0
2	2 M (2) 6 F	21	181.0 $\pm$ 1.0	80.5 $\pm$ 5.5	134 $\pm$ 3	40.6 $\pm$ 1.0
		22	166.2 $\pm$ 1.7	60.2 $\pm$ 1.7		
3	10 F	23	164.8 $\pm$ 2.0	56.5 $\pm$ 1.4	161 $\pm$ 4	46.5 $\pm$ 0.5
4	2 M (1) 8 F	30	178.5 $\pm$ 0.5	70.5 $\pm$ 2.5	132 $\pm$ 6	41.2 $\pm$ 1.4
		24	167.8 $\pm$ 2.3	58.6 $\pm$ 2.1	136 $\pm$ 3	40.0 $\pm$ 0.7
5	3 M 7 F	24	185.0 $\pm$ 7.0	78.7 $\pm$ 7.3	148 $\pm$ 3	45.5 $\pm$ 0.5
		24	165.0 $\pm$ 3.0	58.6 $\pm$ 2.1	133 $\pm$ 3	40.8 $\pm$ 0.9
6	4 M (4) 5 F	36	180.1 $\pm$ 3.7	58.1 $\pm$ 3.9	157 $\pm$ 7	44.0 $\pm$ 1.1
		26	166.6 $\pm$ 1.4	74.3 $\pm$ 4.3	137 $\pm$ 3	40.7 $\pm$ 0.7
7	5 M (3) 5 F	31	183.0 $\pm$ 3.0	54.4 $\pm$ 2.6	155 $\pm$ 7	47.3 $\pm$ 2.0
		22	169.0 $\pm$ 2.0	75.2 $\pm$ 3.1	135 $\pm$ 2	40.6 $\pm$ 0.7
8	1 M (1) 8 F (1)	48	184.0	78.0	147 $\pm$ 1	44.0 $\pm$ 0.5
		32	168.3 $\pm$ 1.1	60.6 $\pm$ 2.5	128 $\pm$ 3	39.2 $\pm$ 1.2
9	3 M (3) 7 F	34	180.0 $\pm$ 3.8	76.0 $\pm$ 8.5	166	48
		26	167.1 $\pm$ 1.9	55.0 $\pm$ 1.0	131 $\pm$ 2	40.6 $\pm$ 0.7
10	3 M (1) 6 F (2)	30	174.7 $\pm$ 2.7	77.3 $\pm$ 9.2	157 $\pm$ 3	47.0 $\pm$ 1.0
		29	166.3 $\pm$ 1.7	55.7 $\pm$ 2.0	134 $\pm$ 3	40.6 $\pm$ 0.9
11	2 M (2) 8 F (1)	34	179.0 $\pm$ 6.0	76.5 $\pm$ 7.5	162 $\pm$ 7	47.3 $\pm$ 1.8
		23	167.8 $\pm$ 1.7	59.0 $\pm$ 2.1	135 $\pm$ 6	41.0 $\pm$ 2.1
12	3 M (2) 7 F (1)	33	179.7 $\pm$ 5.0	69.0 $\pm$ 0.6	148 $\pm$ 3	43.5 $\pm$ 1.5
		26	167.6 $\pm$ 1.8	61.9 $\pm$ 3.6	130 $\pm$ 4	39.6 $\pm$ 0.7
13	3 M (3) 4 F	37	180.7 $\pm$ 2.3	75.0 $\pm$ 2.6	147 $\pm$ 6	44.0 $\pm$ 1.5
		22	169.0 $\pm$ 3.8	59.3 $\pm$ 6.7	133 $\pm$ 2	40.4 $\pm$ 0.7
14	1 M (1) 8 F (2)	28	178	75	161 $\pm$ 4	47.0 $\pm$ 2.0
		27	167.6 $\pm$ 4.9	59.3 $\pm$ 8.3	131 $\pm$ 9	40.5 $\pm$ 3.3
15	4 M 5 F	25	184.3 $\pm$ 3.8	77.8 $\pm$ 4.4	160	48
		24	171.4 $\pm$ 2.9	61.8 $\pm$ 2.8	132 $\pm$ 6	39.8 $\pm$ 1.9
					153 $\pm$ 4	46.3 $\pm$ 1.2
					134 $\pm$ 4	40.6 $\pm$ 1.0

\* Number of regular blood donors in parentheses.

reference doses. All procedures and methods of calculation have been described previously (9).

Since the  $^{59}\text{Fe}$  added as  $^{59}\text{FeCl}_3$  to the wheat flour uniformly labels the nonheme iron pool in the meals studied, the ratio of the fractions of administered  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  that have been absorbed will be a direct measure of the fraction of the  $^{55}\text{Fe}$  labeled carbonyl iron that joined the nonheme iron pool. This fraction is quite independent of the magnitude of the iron absorption and is a measure of the bioavailability of carbonyl iron in relation to the bioavailability of an easily soluble iron compound, eg ferrous sulfate, known to completely mix with the nonheme iron pool when added as a fortificant. The term relative bioavailability has therefore been used as a synonym for the  $^{55}\text{Fe}/^{59}\text{Fe}$  ratio. In one study (no 13), breakfast meals fortified with carbonyl iron and labeled with  $^{55}\text{Fe}$  were served

on two mornings and another breakfast meal, fortified with the same amount of iron as ferrous sulfate and labeled with  $^{59}\text{Fe}$ , was served on the next two mornings on four consecutive days.

As a basis of comparison, the absorption of iron was also measured from the breakfast meal and the hamburger meal when no extra iron had been used to fortify the wheat rolls. In these unfortified meals the iron absorption was measured both with and without the addition of 50 mg of crystalline ascorbic acid. In these latter two studies, four meals were served to each subject: two meals with and two without ascorbic acid, the meals being labeled with two different radioiron isotopes.

Three levels of iron fortification were studied. In each meal, wheat rolls made from 40 g of unfortified white wheat flour were served. The amounts of carbonyl iron

TABLE 2  
Content of native iron and fortification iron in meals studied and absorption of iron from these iron sources

Study meal	N	Nonheme iron content (mg)		Total	Absorption %		Relative bioavailability $^{59}\text{Fe}/^{56}\text{Fe}$ mean $\pm$ SEM	Reference dose absorption %
		Native	Added		Native iron $^{59}\text{Fe}$	Added iron $^{59}\text{Fe}$		
1. Breakfast with coffee	10	0.4	1	1.4	5.6	1.0	0.20 $\pm$ 0.03	40.6
2. Hamburger meal	8	3.4	1	4.4	12.7	1.7	0.11 $\pm$ 0.02	47.3
3. Breakfast with milk	10	0.5	1	1.5	5.5	0.5	0.09 $\pm$ 0.01	26.4
4. Meat soup and bread	10	1.3	1	2.3	20.1	1.1	0.05 $\pm$ 0.005	31.9
5. Breakfast with coffee	10	0.4	2	2.4	5.2	0.9	0.23 $\pm$ 0.05	32.7
6. Breakfast with coffee	9	0.4	3	3.4	8.2	1.8	0.25 $\pm$ 0.04	32.9
7. Hamburger meal	10	3.4	2	5.4	12.1	1.6	0.13 $\pm$ 0.01	32.4
8. Breakfast with coffee with 25 mg ascorbic acid	9	0.4	1	1.4	9.0	1.8	0.22 $\pm$ 0.05	45.5
9. Breakfast with coffee with 25 mg ascorbic acid	10	0.4	1	1.4	6.8	1.2	0.23 $\pm$ 0.04	41.1
10. Breakfast with coffee with 50 mg ascorbic acid	9	0.4	2	2.4	9.8	2.2	0.33 $\pm$ 0.08	36.9
11. Hamburger meal with 50 mg ascorbic acid	10	3.4	1	4.4	12.7	2.2	0.15 $\pm$ 0.04	32.9
12. Breakfast: Wheat flour porridge and coffee	10	0.4	1	1.4	4.3	0.7	0.17 $\pm$ 0.02	50.4
13. Breakfast with coffee (Comparison: Carbonyl iron $^{59}\text{FeSO}_4$ )	7	0.4	1	1.4	11.1	2.7	0.24 $\pm$ 0.04	38.8
14. Breakfast (unfortified bread) with coffee with/without 50 mg ascorbic acid	9	0.4	—	0.4	6.0	9.2	Ratio 1.68 $\pm$ 0.24	44.4
15. Hamburger (unfortified bread) with/without 50 mg ascorbic acid	9	3.4	—	3.4	8.5	12.7	1.55 $\pm$ 0.13	25.5

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included in the rolls were 1, 2, or 3 mg which corresponds to levels of iron fortification of 2.5, 5.0, and 7.5 mg iron per 100 g of wheat flour.

#### Meal composition and preparation

The rolls in all the studies were made from 40 g of unfortified wheat flour of 60% extraction. The native iron content was 0.4 mg. Weighed amounts of labeled carbonyl iron and cold carbonyl iron were carefully mixed with 20 mg wheat flour in a glass beaker. This labeled flour (premix) was then mixed with the rest of the flour needed and used for several studies. This procedure was necessary to ensure a uniform distribution of the carbonyl iron in the flour.

The dough was fermented for 30 min at 23°C. The dough was kneaded and weighed amounts transferred to small aluminum forms which were left standing for 10 min for further fermentation. The bread was baked at 250°C for 15 min.

The coffee breakfast meal consisted of one wheat roll with margarine (12 g), orange marmalade (10 mg), and coffee (150 ml). The milk breakfast meal consisted of one wheat roll with margarine (12 g) and 150 ml of milk (3% fat). The meat soup meal consisted of beef broth made from 20 g of beef and 250 ml of vegetable broth containing 70 g carrots, white cabbage, and parsley (a commercial product, Gustav Bong AB). One wheat roll with margarine (12 g) was served with the soup. The hamburger meal consisted of hamburgers (110 g), string beans (60 g), and mashed potatoes (150 g). The hamburger served was a commercial product containing 66 g of minced meat, which has been used in previous iron absorption studies (10). One wheat roll with margarine (12 g) was served with the meal.

In one of the breakfast meals, the wheat flour was boiled to porridge with 250 ml of milk (3% fat), sugar (13 g), and margarine (10 g). The porridge was served together with 25 g of jam and 150 ml of coffee.

In studies 1-12, two-duplicate meals were served, labeled with 1.5  $\mu\text{Ci}$   $^{59}\text{Fe}$  and 2  $\mu\text{Ci}$   $^{55}\text{Fe}$ . In studies 13-15 two meals were labeled with 1.5  $\mu\text{Ci}$   $^{59}\text{Fe}$  each, and two meals with 2  $\mu\text{Ci}$   $^{55}\text{Fe}$  each.

#### Chemical composition of meals

Aliquots of the meals were freeze-dried and then finely ground to a powder in a porcelain mortar. Weighed amounts of this powder were analyzed for total iron (11), nonheme iron (9), and phytic acid (12). The chemical composition of the meals is shown in Table 3.

#### Oral reference doses of iron

A solution of 10 ml of 0.01 M hydrochloric acid containing 3 mg iron as ferrous sulfate and 30 mg ascorbic acid was used as a reference in all studies. The 10-ml vials containing the iron solution were rinsed twice with water that was also consumed. Each subject received two reference doses on two consecutive mornings after an overnight fast. No food or drink was allowed after the reference dose for 3 h. Each subject received a total of 1.5 ml  $\mu\text{Ci}$   $^{59}\text{Fe}$ .

#### Preparation of radioiron labeled carbonyl iron

One thousand two hundred milligrams of carbonyl iron powder (BASF) was irradiated in vacuum in a quartz glass

TABLE 3  
Chemical composition of the meals

Meal	Energy kcal	Protein g	Phytic P mg
<i>Breakfast:</i>			
Wheat buns, margarine, marmalade, coffee	260	4	0
<i>Breakfast:</i>			
Wheat flour porridge, jam, coffee	395	12	23
<i>Breakfast:</i>			
Wheat buns, margarine, milk	385	9	0
<i>Hamburger meal:</i>			
Hamburgers, mashed potatoes, string beans, wheat buns, margarine	680	23	0
<i>Meat soup meal:</i>			
Meat soup with vegetables, wheat buns, margarine	350	8	0

ampoule at Edig Institut für Reaktorforschung, Würenlingen, Schweiz. The neutron flow was  $1.5 \times 10^{13}$  n/cm<sup>2</sup> s and the integrated neutron flow  $1.426 \times 10^{19}$  nut. The temperature never exceeded 100°C. At the end of the irradiation period (261 h) the sample contained 4.2 mCi  $^{59}\text{Fe}$  and 1.9 mCi  $^{55}\text{Fe}$ .

The sample was left standing to allow a decay of  $^{59}\text{Fe}$  and other possible contaminating radioisotopes for a period of at least 500 days thus reducing the  $^{59}\text{Fe}$  activity to <1/1000 of the original activity whereas the  $^{55}\text{Fe}$  activity had only decayed with about 30%. The energy spectrum of the irradiated sample of carbonyl iron was studied in a 3 inch NaJ scintillation well counter. Besides the mentioned  $^{59}\text{Fe}$  activity,  $^{54}\text{Mn}$  activity could also be detected. An amount of 2  $\mu\text{Ci}$   $^{55}\text{Fe}$  was contaminated with 0.003  $\mu\text{Ci}$   $^{59}\text{Fe}$  and 0.003  $\mu\text{Ci}$   $^{54}\text{Mn}$  at the time of the first studies in this paper. Due to this very low contamination the  $^{55}\text{Fe}$  labeled carbonyl iron gave no measurable activity above background in the high-energy ( $^{59}\text{Fe}$ ) window.

#### Measurement of dissolution rate

The dissolution rate was studied in hydrochloric acid at pH 1 and pH 3. Weighed amounts of the compounds, corresponding to 15 mg elemental iron and 200 ml 0.1 N HCL (or 0.001 N HCL) were shaken in a 600 ml flask at 37° using horizontal agitation (amplitude 60 mm frequency 130 cycles/min). Duplicate 2 ml samples were withdrawn at various intervals. The samples were immediately filtered through acid-washed, fine-pore filter paper (Whatman OOH) and the filtrate was immediately again filtered through a Millipore<sup>R</sup> filter [Millex-GS (0.22  $\mu$ )]. The Fe content of the filtrate was determined using a bathophenanthroline method (13).

## Results

### *The relative bioavailability of carbonyl iron given with different meals*

As shown in Table 2 and Figure 1, the relative bioavailability of the carbonyl iron was very different in different meals. In a continental type of breakfast with coffee (study 1), 5.6% of the native iron was absorbed but only 1% of the carbonyl iron. The mean value of the individual absorption ratio values was 0.20. This figure thus represents the relative bioavailability of the carbonyl iron and implies that 20% of the carbonyl iron joined the non-heme pool. If the same wheat rolls fortified with carbonyl iron were served with a hamburger meal the relative bioavailability was lower ( $p < 0.001$ ), only 11%, but due to the higher absorption of nonheme iron from the hamburger meal, about 2% of the added carbonyl iron was absorbed. If the breakfast was served with milk, instead of coffee, the relative bioavailability of the carbonyl iron decreased from 20 to 9% ( $p < 0.001$ ) and when the fortified wheat rolls were given with a meat soup, the relative bioavailability fell to 5%.

### *Relative bioavailability of carbonyl iron in relation to amount given*

Three levels of iron fortification were studied with the breakfast-coffee meals corresponding to fortification levels of 2.5, 5.0, and 7.5 mg carbonyl iron per 100 g of wheat flour (1, 2, or 3 mg of iron was added to the 40 g of flour used for baking the rolls served with each meal).

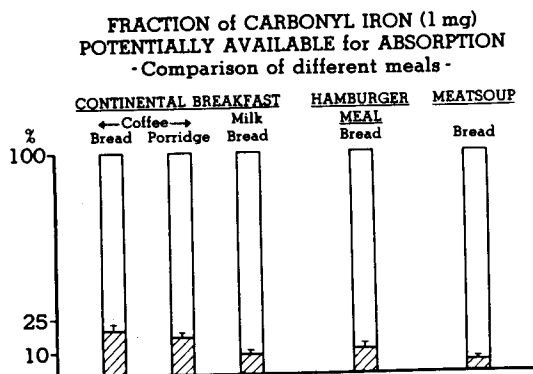


FIG 1. Relative bioavailability of carbonyl iron when 1 mg baked in wheat rolls was given with different meals. In one meal the fortified wheat flour was not baked to bread but given as a wheat porridge.

For the three levels of iron fortification (study 1, 5, 6) the relative bioavailability of the carbonyl iron was  $0.20 \pm 0.03$ ;  $0.23 \pm 0.05$ , and  $0.25 \pm 0.04$ , respectively. The differences between the ratios are not statistically significant.

With the hamburger meal, two levels of iron fortification were studied (1 or 2 mg carbonyl iron was added: study 2 and 7). The relative bioavailability of the two dose levels was essentially the same,  $0.11 \pm 0.02$  and  $0.13 \pm 0.01$ , respectively.

### *Does fermentation and baking affect the bioavailability of carbonyl iron?*

To answer this question, a comparison was made between study 1 and 12. In both studies 1 mg carbonyl iron was added to the meals which were identical in composition except for the baking. In study 12 the same amount of fortified wheat flour was used but served as a porridge. The difference between the values for relative bioavailability in the two studies was not statistically significant—the mean values being  $0.20 \pm 0.03$  and  $0.17 \pm 0.02$  ( $\bar{x} \pm \text{SEM}$ ), respectively. Thus we conclude that fermentation and baking did not change the relative bioavailability of carbonyl iron.

### *Does fermentation and baking affect the bioavailability of a soluble iron salt ( $\text{FeSO}_4$ ) more than of carbonyl iron?*

Breakfast meals were fortified either with 1 mg of carbonyl iron labeled with  $^{55}\text{Fe}$  or with 1 mg of iron as ferrous sulfate labeled with  $^{59}\text{Fe}$  (study 13). These two kinds of breakfast meals, A and B, were given on four consecutive mornings in the order ABBA and BAAB. The absorption ratio of the tracers was  $0.24 \pm 0.04$  which is the same ratio as the ratio of the absorption of iron from the carbonyl iron and the total absorption from the nonheme iron pool of the same breakfast meals in study 1 (ie the relative bioavailability of carbonyl iron). Thus, the baking process did not impair the bioavailability of iron given as ferrous sulfate or as carbonyl iron.

### *Does addition of ascorbic acid affect the relative bioavailability of carbonyl iron?*

In these studies (no 8–11) the ascorbic acid was added just before serving to prevent any destruction during preparation of the meals.

Breakfast meals were given with or without 25 or 50 mg of ascorbic acid. The rolls in each meal contained 1 mg of carbonyl iron. The relative bioavailabilities did not change with the addition of ascorbic acid and were  $0.20 \pm 0.03$  without ascorbic acid, and  $0.22 \pm 0.06$  and  $0.23 \pm 0.04$  with 25 and 50 mg ascorbic acid, respectively. With the dose level of 2 mg carbonyl iron and 50 mg ascorbic acid, the relative bioavailability was  $0.33 \pm 0.08$  which was higher than the figure  $0.23 \pm 0.05$  in study 5 in which also 2 mg carbonyl iron was given without ascorbic acid. This difference, however, was not statistically significant. The relative bioavailability of carbonyl iron was also unchanged when the fortified bread was given in the hamburger meal. Without ascorbic acid, the relative bioavailability was  $0.11 \pm 0.02$  at the 1 mg level and  $0.13 \pm 0.01$  at the 2 mg level. With ascorbic acid the relative bioavailability was  $0.15 \pm 0.04$  at the 1 mg level of added carbonyl iron.

*Solubility studies of carbonyl iron and another elemental iron powder (Glidden A 131) in hydrochloric acid*

The rate of dissolution of iron was studied in the carbonyl iron that had been irradiated and in nonirradiated samples of carbonyl iron from the same batch. A freshly prepared sample of the same kind of carbonyl iron from the same manufacturer was also included. As shown in Table 4, the same rate of dissolution was observed in all preparations, at pH 1 and pH 3. The rate of dissolution of another elemental iron powder (Glidden A 131) was about the same as that of carbonyl iron, both at pH 1 and 3. It was included in the present study as it is a commonly used form of ele-

mental iron for iron fortification of bread and cereals, and to have one further known iron preparation as a reference in the system used to measure rate of dissolution.

### Discussion

The main findings of the present studies were consistent and clear-cut: The relative bioavailability of carbonyl iron added to wheat flour and served as bread 1) was much lower than would be expected from animal studies, 2) did not vary with levels of iron fortification usually employed, 3) was not affected by the addition of ascorbic acid, 4) was very much influenced by the composition of the meal containing the fortification iron, and 5) was not affected by the fermentation and the baking of the bread.

The relative bioavailability varied very little in the different studies when the same meal was given. The probable main reason is that the marked variation in iron absorption between subjects and the variation in absorption in the same subject, studied on different days, do not affect the *ratio* between the absorption of carbonyl iron in the nonheme iron pool and the absorption of native iron in the nonheme iron pool. The main source of variation in the present study is probably related to differences in rate and extent of dissolution of the carbonyl iron in the gastrointestinal tract.

No obvious reason can be given why the relative bioavailability of carbonyl iron is much lower in man than would be anticipated from animal studies. In animal assays, made mainly in iron depleted rats, carbonyl iron has been shown to be more efficacious than other elemental iron powders (6) and in one study in rats it was found to be equally efficacious as ferrous sulfate when baked into bread (7). There are reasons to believe that there can be marked species differences in iron absorption. Iron absorption in the rat, for example, is about 100 times higher per unit body weight than in man (14). The possibility that the neutron irradiation of the carbonyl iron powder had led to an impairment of its relative bioavailability was carefully considered, however. An impairment of practical importance was excluded by the finding of identical dissolution rate curves of the irradiated and the nonirradiated carbonyl iron from the same batch. A negative effect of storage could also be ex-

TABLE 4  
Rate of dissolution (percent dissolved at different times - minutes) at pH 1 and 3 of the carbonyl iron studied and as a comparison a batch of another elemental iron powder (Glidden A 131)

	pH 1				pH 3
	10'	20'	30'	60'	30'
<i>Carbonyl iron</i>					
Original batch	36	65	85	100	6.0
- irradiated	38	63	83	100	6.1
New batch	32	62	82	96	6.0
<i>Glidden A-131</i>	42	71	85	99	5.9

cluded as the dissolution rate was the same for the original sample and a new sample of carbonyl iron (Table 4).

In *in vitro* studies it has been observed that during baking, large amounts of insoluble forms of iron are produced and this conversion was independent of the iron compound used, being the same for example, for ferrous sulfate and for elemental iron powders, including carbonyl iron (15). Two sets of observations in the present study, however, do not confirm these findings. Firstly, the observation that the bioavailability of ferrous sulfate added as a fortificant to flour was about four times better absorbed than the carbonyl iron. Secondly, the fact that the relative bioavailability of carbonyl iron was the same when iron fortified flour was baked into bread and when served as a wheat porridge.

The addition of ascorbic acid did not increase the *relative* bioavailability of carbonyl iron. It must be emphasized, however, that the bioavailability of the fraction of carbonyl iron that is dissolved and thus joins the nonheme iron pool of the gastrointestinal content is absorbed much better when ascorbic acid is present.

A main and unexpected finding was the marked difference in the relative bioavailability of the carbonyl iron when it was included in different meals. In one way or the other, the difference in relative bioavailability must be related to differences in the rate of dissolution of the carbonyl iron. As this rate is very dependent on pH and time, there may be two main factors explaining these results. Differences in pH of the gastric content of the different meals may thus be one factor determining the rate of dissolution. Another explanation would be differences in the rate of gastric emptying. It is known that the acidity of the gastric content is higher in a meal such as the breakfast served with coffee than in meals served without coffee and with a greater buffering capacity related to the proteins present in meat and milk. It is therefore difficult to predict the average relative bioavailability of the carbonyl iron in the diet as a whole, as the properties of the meals containing most of the fortification iron will markedly influence the average figure. It is reasonable to estimate that in Western type diets where the bread is the main vehicle for fortification iron, about

10-15% of the carbonyl iron would join the nonheme iron pool from which a certain fraction will then be absorbed. The 85-90% of carbonyl iron not joining the pool, could thus be considered as wasted.

In an earlier study on a series of experimentally produced preparations of radioiron labeled elemental iron powders, we found a strong relationship between the rate of dissolution (studied in the same way as in the present paper) and the relative bioavailability in man (16).

In earlier animal studies and *in vitro* studies, carbonyl iron was found to have the highest solubility of several commercial iron powders (6) and the highest bioavailability in animal tests (6, 7). With the method we used to study rate of dissolution we found that the hydrogen-reduced iron powder (Glidden A 131) had about the same rate of dissolution as the carbonyl iron we used. Thus the balance of evidence, from our studies and others, indicates that the bioavailability of good commercial iron powders are relatively poor in man. Two experimental produced iron powders have been shown to have a higher bioavailability in man (16, 17). However, it is doubtful if these products will ever be commercially available, since very small particle size products or products with a very large reactive surface area are difficult to produce and difficult to handle safely due to their pyrophoric property (burning to incandescence when exposed to air).

The present studies clearly show that the results of the hemoglobin repletion method in animals can lead to quite misleading conclusions as to the bioavailability of iron fortificants in humans. While animal assays and/or physicochemical tests, such as standardized dissolution rate measurements, are needed for the production control of iron compounds to be used for iron fortification in man, such procedures must be carefully standardized and validated by comparisons with bioavailability studies in man made under as realistic conditions as possible. These conditions should include studies made with meals usually providing most of the fortification iron.

The present findings that carbonyl iron has a low and variable bioavailability in man, and a critical evaluation of previous studies made on other forms of reduced iron, makes it necessary to reconsider the rationale of using el-

emental iron powders for the fortification of foods for human consumption. It is important to look for other compounds with good technical properties and with a known, higher, and less variable bioavailability in man. □

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### References

1. Vyas D, Chandra RK. Functional implications of iron deficiency. In: Stekel A, ed. Iron nutrition in infancy and childhood. New York, NY: Raven Press, 1984;45-59.
2. Lee K, Clydesdale FM. Iron sources used in food fortification and their changes due to food processing. *Crit Rev Food Sci Nutr* 1979;11:117-53.
3. Cook JD, Reusser ME. Iron fortification; an update. *Am J Clin Nutr* 1983;38:648-59.
4. Hurrell RF. Bioavailability of different iron compounds used to fortify formulas and cereals: technological problems. In: Stekel A, ed. Iron nutrition in infancy and childhood. New York, NY: Raven Press, 1984;147-76.
5. Pla GW, Fritz JC, Rollinson CL. Relationship between the biological availability and solubility rate of reduced iron. *J Assoc Off Anal Chem* 1976;59:582-3.
6. Shah BG, Giroux A, Belonje B. Specifications for reduced iron as a food additive. *J Agric Food Chem* 1977;25:592-4.
7. Sacks PV, Houchin DN. Comparative bioavailability of elemental iron powders for repair of iron deficiency anemia in rats. Studies of efficacy and toxicity of carbonyl iron. *Am J Clin Nutr* 1978;31:566-73.
8. Hallberg L. Iron nutrition and food iron fortification. *Seminars in Hematology* 1982;19:31-47.
9. Hallberg L. Food iron absorption. In: Cook JD, ed. *Methods in hematology*. London: Churchill, 1980: 116-33.
10. Hallberg L, Rossander L. Absorption of iron from Western-type lunch and dinner meals. *Am J Clin Nutr* 1982;35:502-9.
11. Björn-Rasmussen E, Hallberg L, Isaksson B, Arvidsson B. Food iron absorption in man. Application of the two-pool extrinsic tag method to measure heme and nonheme iron absorption from the whole diet. *J Clin Invest* 1974;53:247-55.
12. Nordic Committee on Food Analysis (NCFA). Method No 17:2. Skelbaecksgade, Köpenhamn V: Danish Technical Press, 1966.
13. International Committee for Standardization in Hematology (Iron Panel). The measurement of total and unsaturated iron-binding capacity in serum. *Br J Haematol* 1978;38:281-90.
14. Finch CA, Ragan HA, Dyer IA, Cook JD. Body iron loss in animals. *Proc Soc Exper Biol Med* 1978;159: 335-8.
15. Lee K, Clydesdale FM. Effect of baking on the forms of iron in iron-enriched flour. *J Food Sci* 1980;45: 1500-4.
16. Björn-Rasmussen E, Hallberg L, Rossander L. Absorption of fortification iron. Bioavailability in man of different samples of reduced iron, and prediction of the effects of iron fortification. *Br J Nutr* 1977;37: 375-88.
17. Cook JD, Minnick V, Moore CV, Rasmussen A, Bradley WB, Finch CA. Absorption of fortification iron in bread. *Am J Clin Nutr* 1973;26:861-72.