

# *Helicobacter pylori* infection, iron absorption, and gastric acid secretion in Bangladeshi children<sup>1-4</sup>

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## ABSTRACT

**Background:** Nonheme-iron absorption requires an acidic milieu. Reduced gastric acid output as a consequence of *Helicobacter pylori* infection could be an important limiting factor for iron absorption.

**Objective:** We measured gastric acid output and iron absorption from a non-water-soluble iron compound (ferrous fumarate) and a water-soluble iron compound (ferrous sulfate) in children with and without *H. pylori* infection.

**Design:** Gastric acid output was quantified before (basal acid output, or BAO) and after pentagastrin stimulation (stimulated acid output, or SAO) in 2–5-y-old children with iron deficiency anemia who were ( $n = 13$ ) or were not ( $n = 12$ ) infected with *H. pylori*. Iron absorption was measured by using a double-stable-isotope technique. *H. pylori*-infected children were studied before and after eradication therapy.

**Results:** BAO and SAO were significantly lower in the *H. pylori*-infected children ( $0.2 \pm 0.2$  and  $1.6 \pm 0.9$  mmol/h, respectively) than in the uninfected children ( $0.9 \pm 0.7$  and  $3.1 \pm 0.9$  mmol/h, respectively;  $P = 0.01$  and  $P < 0.005$ ). BAO and SAO improved to  $0.8 \pm 1.3$  and  $3.3 \pm 2.4$  mmol/h, respectively, after therapy. Iron absorption from ferrous sulfate was significantly greater than that from ferrous fumarate both before (geometric  $\bar{x}$ : 19.7% compared with 5.3%;  $P < 0.0001$ ) and after (22.5% compared with 6.4%;  $P < 0.0001$ ) treatment in *H. pylori*-infected children. Corresponding values for uninfected children were 15.6% and 5.4%, respectively ( $P < 0.001$ ;  $n = 12$ ).

**Conclusions:** Iron absorption from ferrous fumarate was significantly lower than that from ferrous sulfate in both *H. pylori*-infected and uninfected Bangladeshi children. Treatment of *H. pylori* infection improved gastric acid output but did not significantly influence iron absorption. The efficacy of ferrous fumarate in iron fortification programs to prevent iron deficiency in young children should be evaluated. *Am J Clin Nutr* 2004;80:149–53.

**KEY WORDS** *Helicobacter pylori*, gastric acid secretion, iron absorption, anemia, ferrous fumarate, iron status, children

## INTRODUCTION

Iron deficiency is a major public health problem, especially in infants, children, and women of childbearing age in developing countries (1, 2). The consequences of iron deficiency anemia (IDA) are particularly significant in infants and young children and include abnormalities of immune function, poor growth, and potentially irreversible deficits of cognition and motor function (2).

Low dietary intake of poorly bioavailable iron is believed to be the principal cause of IDA in the developing world. Dietary iron in resource-poor areas is predominantly nonheme iron of plant origin, which contains high amounts of inhibitors of iron absorption, such as phytate (3). Gastric acid secretion is also an important intraluminal factor for nonheme-iron absorption (4, 5). Ingested dietary ferric ( $\text{Fe}^{3+}$ ) iron is solubilized and ionized by gastric acid and reduced to the more readily absorbed ferrous ( $\text{Fe}^{2+}$ ) form. Conditions affecting gastric acid secretion are therefore potentially important factors in the etiology of IDA (6).

*Helicobacter pylori* infection is the most common infection worldwide. Its prevalence is very high in developing countries, such as in Bangladesh, where  $\approx 60\%$  of children aged  $<5$  y are infected (7). Infection is typically acquired in childhood and persists throughout life, causing chronic gastritis, a risk factor for gastric atrophy and gastric cancer (8). Among infected children who have undergone endoscopy, chronic gastritis is a near universal finding (9, 10). An important consequence of chronic *H. pylori* gastritis and gastric atrophy is low gastric acid output (11). Low gastric acid secretion results in an impaired “gastric barrier,” which is associated with increased susceptibility to enteric infections, a major public health concern linked to diarrhea, malnutrition, and growth failure in children in the developing world (12, 13). Several reports have indicated an association between *H. pylori* infection and anemia, iron deficiency, and IDA, although the nature of the interactions has not been established (14–17).

Iron fortification of foods is considered among the most cost-effective approaches to preventing iron deficiency (18).

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<sup>2</sup> Supported by a grant from the National Institutes of Health (DK 53032-02) and by the Swiss Federal Institute of Technology, Zurich, Switzerland.

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Received October 8, 2003.

Accepted for publication December 29, 2003.

Non-water-soluble iron compounds are often used in fortification programs because they cause no unacceptable organoleptic changes in the fortified food. However, these compounds are also poorly soluble in gastric secretions, and the bioavailability of iron is low. Ferrous fumarate is a common fortificant of infant cereals that, because it is soluble in dilute acid but poorly soluble in water, causes less organoleptic changes in the food vehicle than do water-soluble compounds (19, 20). However, if gastric acid output is compromised in a large proportion of the target population, the effect of food fortification programs using ferrous fumarate might be less than expected as a result of a reduced capacity to absorb iron from the fortified food.

The aim of the present study was to measure gastric acid secretion and iron absorption from a non-water-soluble iron compound (ferrous fumarate) and from a water-soluble iron compound (ferrous sulfate) before and after treatment in young Bangladeshi children infected with *H. pylori*. For comparison, uninfected children in the same community were studied in parallel at baseline. All children had IDA.

## SUBJECTS AND METHODS

### Subjects

Iron-deficient anemic children with and without *H. pylori* infection were recruited from Nandipara, a periurban community 7 km from Dhaka City, where the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), maintains a field clinic and has previously conducted studies (7). The prevalence of *H. pylori* infection in children <5 y old in this community is 60% (21). A survey of children in the community was conducted to identify all those meeting the study eligibility criteria. Apparently healthy children aged 2–5 y with a weight-for-age >60% of the National Center for Health Statistics median were selected sequentially from the survey list and were screened for *H. pylori* infection by a [<sup>13</sup>C]urea breath test and for IDA (hemoglobin <110 g/L and serum ferritin <12 µg/L) (22–24). Children with severe anemia (hemoglobin <70 g/L) or apparent infection or inflammatory process—such as fever, cough, or other sign of infection, including ear discharge—were excluded. The children's parents were informed of the study aims and procedures, and written informed consent was obtained from at least one parent. The children (accompanied by their mothers) were admitted for 3 d to the metabolic ward at the ICDDR,B for gastric acid output testing and administration of labeled test meals for measurements of iron absorption. The study protocol was approved by the Ethical and Research Review Committees of the ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh.

### Iron status

Blood was collected by venipuncture, placed in EDTA-containing and plain tubes, and processed in the laboratory within 3 h of collection for measurement of hemoglobin and ferritin. Hemoglobin was quantified by the cyanmethemoglobin method (Sigma, St Louis) and plasma ferritin by enzyme-linked immunosorbent assay (Ramco, Houston). Commercial quality-control materials (Diamed Diagnostics and Medical Products, Cressier s/Morat, Switzerland and Ramco) were analyzed in

parallel. The CVs for the hemoglobin and ferritin quality controls were in the ranges of 1–2% and 2.5–4%, respectively.

### [<sup>13</sup>C]Urea breath test

Breath samples were collected in evacuated tubes for the measurement of baseline <sup>13</sup>C:<sup>12</sup>C isotope ratios after the children had fasted for 2 h. The children first consumed cow milk (25 mL), followed by 100 mg [<sup>13</sup>C]urea (99%) in 25 mL water (Tracer Technologies, Boston) 30 min later. Breath samples were collected 30 min later by using a pediatric mask. Duplicate samples were analyzed for <sup>13</sup>C:<sup>12</sup>C isotope ratios in respiratory carbon dioxide by isotope ratio mass spectrometry at the Department of Medicine and Research, University of Basel, Switzerland. Breath samples ≥3.5 ‰ over baseline (<sup>13</sup>C:<sup>12</sup>C isotope ratios) were regarded as being positive for *H. pylori* infection (21). The [<sup>13</sup>C]urea breath test has been shown to be 100% sensitive and 92% specific and is considered a reference standard for the diagnosis of *H. pylori* infection in children (25).

### Gastric acid secretion

After the children had fasted for 5 h, a soft nasogastric tube was introduced. After aspiration of the resting gastric juice, basal samples were collected for 30 min. Pentagastrin (Cambridge Laboratories, Newcastle on Tyne, United Kingdom) was administered subcutaneously (6 µg/kg), and gastric juice aspiration was continued for an additional 60 min. Samples were collected and stored in 15-min aliquots. The acidity of each sample was measured by titration of 1 mL gastric juice with 0.01 N sodium hydroxide to a pH of 7.4 by using an automated titrator (Metrohm, Herisau, Switzerland). Acid output was calculated for each time point by multiplying the volume of gastric juice by the respective acid concentration. Basal acid output and stimulated acid output were calculated based on 2 samples collected for baseline measurement and the 4 samples collected after the administration of pentagastrin and were expressed as mmol/h.

### Test meal and isotopic labels

Iron absorption from ferrous fumarate was compared with that from a highly bioavailable, water-soluble iron compound (ferrous sulfate) in a randomized crossover study by use of a double stable-isotope technique. Incorporation of <sup>57</sup>Fe and <sup>58</sup>Fe into erythrocytes 14 d after administration was used as an index of iron absorption (26). An infant cereal (Ceresoy; Nestlé, Vevey, Switzerland) based on wheat and soy and without any added iron or ascorbic acid was produced especially for the study. Test meals consisted of 25 g dry cereal mixed with 100 g hot deionized water. Ascorbic acid (molar ratio of 3:1; ascorbic acid relative to added iron) and [<sup>57</sup>Fe]ferrous fumarate or <sup>58</sup>FeSO<sub>4</sub> (10 mg Fe/100 g dry cereal) were added to the test meals before serving. The children were randomly assigned to start with the test meal labeled with <sup>57</sup>Fe or that labeled with <sup>58</sup>Fe. Cereal iron and calcium contents were quantified by electrothermal flame atomic absorption spectroscopy after mineralization by microwave digestion with an HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> mixture and by standard addition technique to minimize matrix effects. Phytic acid content was measured by using an HPLC technique (27).

[<sup>57</sup>Fe]Ferrous fumarate from the same batch used in previous iron absorption studies was used (28, 29). <sup>58</sup>FeSO<sub>4</sub> was prepared from highly enriched <sup>58</sup>Fe dissolved in 0.1 mol H<sub>2</sub>SO<sub>4</sub>/L.

**TABLE 1**  
Baseline characteristics of the study children<sup>1</sup>

	<i>Helicobacter pylori</i> - infected children (n = 9 F, 4 M)	Uninfected children (n = 4 F, 8 M)
Age (mo)	41.0 ± 13 <sup>2</sup>	39.0 ± 14
Weight (kg)	11.6 ± 2.5	11.6 ± 2.7
Height (cm)	88.8 ± 11	87.2 ± 10
Weight-for-age <sup>3</sup>	77.8 ± 7.0	79.5 ± 11.1
Weight-for-height <sup>3</sup>	92.6 ± 5.7	93.8 ± 5.6
Height-for-age <sup>3</sup>	91.6 ± 4.2	90.7 ± 5.9
Hemoglobin (g/L)	99 ± 7	103 ± 7
Serum ferritin (μg/L)		
Geometric $\bar{x}$	4.8	3.9
+ 1 SD	8.2	10.0
- 1 SD	2.8	1.5

<sup>1</sup> There were no significant differences between the groups.<sup>2</sup>  $\bar{x}$  ± SD (all such values).<sup>3</sup> Median percentage of the National Center for Health Statistics reference.

### Analysis of isotopic composition of blood samples

Whole blood was mineralized by microwave digestion, and iron was separated by anion-exchange chromatography following a solvent-solvent extraction step into diethylether. Iron was analyzed by negative thermal ionization mass spectrometry by using  $\text{FeF}_4^-$  molecular ions with a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT, Bremen, Germany) equipped with a multicollector system for simultaneous ion beam detection. We used the negative thermal ionization technique for iron of Walczyk et al (30, 31).

On the basis of the shift of iron isotope ratios in the blood samples and the amount of iron circulating in the body, the amounts of the <sup>57</sup>Fe and <sup>58</sup>Fe labels present in the blood of the children 14 d after administration were calculated according to isotope-dilution principles. Circulating iron was calculated based on blood volume and hemoglobin concentration (32). For calculation of fractional absorption, 90% incorporation of the absorbed iron into red blood cells was assumed (33).

### Study design

Body weight and height were measured and a venous blood sample was drawn into EDTA-coated tubes after the children had fasted for 6–8 h. The blood drawing was followed by intake of the first labeled test meal. The second test meal was fed on the following day under identical conditions. No food or fluid was given for 3 h after the labeled test meals. On day 16, a venous blood sample was drawn at the clinic in Nandipara, and body weight and height were re-measured.

After a second blood sample was collected, *H. pylori*-infected children began a 14-d course of anti-*H. pylori* therapy of amoxicillin (30 mg · kg<sup>-1</sup> · d<sup>-1</sup>), clarithromycin (15 mg · kg<sup>-1</sup> · d<sup>-1</sup>), and omeprazole (20 mg/d) given in 2 divided doses daily by health workers at home. Four weeks after completing the treatment (day 60), the *H. pylori*-infected children were readmitted to the metabolic study ward for the repeat iron absorption, gastric acid output, and [<sup>13</sup>C]urea breath tests. All children were provided therapeutic ferrous sulfate (3 mg · kg<sup>-1</sup> · d<sup>-1</sup>) on completion of the study.

**TABLE 2**  
Gastric acid output before and after stimulation with pentagastrin in *Helicobacter pylori*-infected and uninfected children with iron deficiency anemia<sup>1</sup>

	BAO	SAO
	mmol/h	
<i>H. pylori</i> -infected children before anti- <i>H. pylori</i> treatment (n = 12)	0.2 ± 0.2	1.6 ± 0.9
<i>H. pylori</i> -infected children after anti- <i>H. pylori</i> treatment (n = 12)	0.8 ± 1.3 <sup>2</sup>	3.3 ± 2.4 <sup>2</sup>
Uninfected children (n = 11)	0.9 ± 0.7 <sup>3</sup>	3.1 ± 0.9 <sup>4</sup>

<sup>1</sup> All values are  $\bar{x}$  ± SD. BAO, basal acid output; SAO, stimulated acid output.<sup>2–4</sup> Significantly different from *H. pylori*-infected children before anti-*H. pylori* treatment: <sup>2</sup>P < 0.05, <sup>3</sup>P = 0.01, <sup>4</sup>P < 0.005.

### Data analyses

Paired and unpaired *t* tests were used to evaluate results within and between the 2 groups. Wilcoxon's signed-ranks test was used to compare paired observations, such as acid outputs before and after treatment, when values were not normally distributed. Iron absorption and plasma ferritin were logarithmically transformed before analysis, and the results are presented as geometric means (+1 SD, -1 SD). All other results are presented as means ± SDs. SPSS (version 8 for WINDOWS; SPSS Inc, Chicago) was used to perform the statistical analyses.

### RESULTS

Twenty-five children (13 with *H. pylori* infection and 12 uninfected) with IDA entered the study. In the infected group, incomplete intake of the labeled test meals resulted in the exclusion of one child at baseline and one child after treatment. One *H. pylori*-infected child was lost to follow-up after treatment because of migration from the community. The baseline characteristics of the children in the 2 groups were not significantly different (**Table 1**). Of the 12 *H. pylori*-infected children, 10 had a negative result on the urea breath test after treatment. The infant cereal contained 2.0 ± 0.02 mg Fe, 45.0 ± 0.3 mg Ca, and 0.41 g phytic acid per 100 g cereal product.

Gastric acid output was significantly lower in *H. pylori*-infected children than in uninfected children (**Table 2**). Both basal and stimulated acid output improved after *H. pylori* eradication therapy and reached amounts not significantly different from those of uninfected children.

Geometric mean iron absorption from ferrous sulfate and ferrous fumarate was 19.7% and 5.3% (*P* < 0.0001; *n* = 12) before treatment and 22.5% and 6.4% after treatment (*P* < 0.0001; *n* = 11) in *H. pylori*-infected children (**Table 3**). Corresponding values for uninfected children were 15.6% and 5.4% (*P* < 0.001; *n* = 12). Geometric mean relative absorption (absorption of ferrous fumarate compared with that of ferrous sulfate) was 26.9% and 34.8% in *H. pylori*-infected and uninfected children, respectively, and 28.3% in *H. pylori*-infected children after treatment. *H. pylori* eradication therapy did not significantly influence iron absorption from ferrous sulfate or ferrous fumarate (*P* = 0.34; *n* = 10).

Hemoglobin improved significantly in the *H. pylori*-infected children with treatment; mean hemoglobin increased from 99 ± 7 to 109 ± 5 g/L after treatment (*P* < 0.005), whereas there was

TABLE 3

Fractional iron absorption from ferrous fumarate and ferrous sulfate in uninfected children with iron deficiency anemia (IDA) and in *Helicobacter pylori*-infected children with IDA before and after treatment

	<i>H. pylori</i> -infected children before treatment (n = 12)			<i>H. pylori</i> -infected children after treatment (n = 11)			Uninfected children (n = 12)		
	Ferrous fumarate	Ferrous sulfate	Relative absorption	Ferrous fumarate	Ferrous sulfate	Relative absorption	Ferrous fumarate	Ferrous sulfate	Relative absorption
		%			%			%	
Geometric $\bar{x}$	5.3	19.7	26.9	6.4	22.5	28.3	5.4	15.6	34.8
+1 SD	13.5	32.9	49.0	12.9	33.0	47.7	12.7	30.1	64.3
-1 SD	2.1	11.8	14.8	3.2	15.4	16.8	2.3	8.1	18.8
$P^1$		<0.0001			<0.0001			<0.001	

<sup>1</sup>  $P$  value for iron absorption from ferrous fumarate compared with that from ferrous sulfate within each group.

no significant difference in hemoglobin over the same period in the uninfected children ( $104 \pm 7$  and  $108 \pm 7$  g/L;  $P > 0.05$ ).

## DISCUSSION

A prominent finding in the present study was that *H. pylori* infection was associated with impaired gastric acid secretion, although the reduced gastric acid secretion did not significantly influence iron absorption from the 2 iron compounds evaluated in this study. No statistically significant improvement in iron absorption of either ferrous sulfate or ferrous fumarate was observed after *H. pylori* eradication therapy despite improvement in gastric acid secretion. Our results do not support the hypothesis that *H. pylori* infection influences iron absorption from water-soluble or non-water-soluble iron compounds. Our study population was relatively young (2–5 y), however, and it is conceivable that the effect of *H. pylori* infection on gastric acid secretion and iron absorption is more pronounced after long-term exposure to the infection.

Although *H. pylori* infection has been associated with iron deficiency and IDA, the mechanism of causality is poorly defined (14–16). Our results indicate that *H. pylori* infection per se does not influence iron absorption in young children. The statistically significant increase in hemoglobin concentration after eradication therapy suggests an important role of *H. pylori* infection in the etiology of anemia. Although we could not demonstrate an influence on iron absorption in the present study, the increase in gastric acid output may have resulted in an improved absorption of native food iron. It is possible that *H. pylori* competes with the host for iron absorption because the organism contains an iron binding protein similar to ferritin and a system of iron-repressive outer membrane proteins with binding activity for heme iron (34, 35). Yet this is unlikely to explain the observations in our children because heme iron intake is negligible in this study population.


A beneficial role of antibiotics in eradicating a coexistent, subclinical infection or in reducing the inflammatory response in association with *H. pylori* infection is another possibility, because anemia can be caused by infection or by general inflammatory disorders through an effect on iron metabolism (36). The systemic inflammatory response to *H. pylori* infection is probably mild but has not been evaluated as a potential cause of *H. pylori*-associated anemia. Gastrointestinal blood loss was reported in association with *H. pylori* infection in one study, but other studies have not confirmed this, and we have not observed

gastrointestinal blood loss in *H. pylori*-infected children in Bangladesh (15; S Sarker and GJ Fuchs, unpublished observations, 2003). Although the mechanism of *H. pylori* infection in the etiology of anemia and IDA remains to be fully defined, it is likely that this infection should be considered together with gastrointestinal parasites (such as hookworm), malaria, and other infections when developing public health strategies to combat anemia and IDA.

The second major finding of the current study was that iron absorption from ferrous fumarate was significantly lower than that from ferrous sulfate. Its mean absorption relative to ferrous sulfate in both *H. pylori*-infected and uninfected children was only 27–35% compared with 100% in Western adults (19, 20). Although gastric acid secretion improved after anti-*H. pylori* treatment, iron absorption was not influenced. These results indicate that gastric acid output after treatment was still too low to optimally solubilize ferrous fumarate. Gastric acid secretion in our uninfected children and *H. pylori*-infected children post-treatment was similar to that in apparently healthy children in Bangladesh and the United States (37, 38). It can therefore be expected that the low relative iron absorption from ferrous fumarate observed in the present study is not specific to children living in developing countries but can also be expected in healthy Western children. It should be emphasized that the Bangladeshi children had IDA and were less well nourished than are Western children and that the influence of these conditions on the results is not known.

Only limited information is available on iron absorption from non-water-soluble iron compounds in young children, and no data on iron absorption from ferrous fumarate have been reported in 2–5-y-old children living in industrialized countries. The labeled ferrous fumarate compound used in the present study has been previously evaluated and found to be significantly better absorbed than ferric pyrophosphate in European infants (28). Although the labeled compound was not previously compared directly against ferrous sulfate in children, recent data from Guatemala indicate that iron absorption from ferrous fumarate and ferrous sulfate is similar in 12-y-old children (29).

We conclude that gastric acid output was impaired in *H. pylori*-infected children compared with that in uninfected children and that treatment of *H. pylori* infection improved gastric acid output and hemoglobin concentrations but did not significantly influence iron absorption. Furthermore, contrary to observations in healthy Western adults, iron absorption from ferrous fumarate was significantly lower than that from ferrous sulfate in both *H.*

*pylori*-infected and uninfected Bangladeshi children. The effect of iron fortification programs that use ferrous fumarate or other non-water-soluble iron compounds to prevent iron deficiency in children such as ours should be defined. 

We gratefully acknowledge the participation in the present study of the children and their parents. We thank Pius Hildebrand, University of Basel, for analysis of breath samples (urea breath test); Christophe Zeder, Swiss Federal Institute of Technology, Zurich, for analysis of iron stable isotopes in blood samples and food analyses; MA Wahed for performing the biochemical iron status assays; Rekha Chanda (ICDDR,B) for excellent coordination of the fieldwork; and the nurses in the metabolic ward at the ICDDR,B for their care.

SAS was responsible for the design of the study, data collection and analysis, and writing of the manuscript; LD was responsible for the design of the study, data collection and analysis, and writing of the manuscript; HM was responsible for data collection; TW was responsible for data collection and analysis and manuscript review; RFH was responsible for the design of the study and manuscript review; NG was responsible for data collection and analysis and manuscript review; and GJF was responsible for the design of the study, data analysis, and writing of the manuscript. None of the authors had a conflict of interest with any of the organizations sponsoring the research, including advisory board affiliations.

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