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Human Nutrition and Metabolism **Research Communication**

Heme-Iron Absorption Is Saturable by Heme-Iron Dose in Women¹

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ABSTRACT In developed countries where meat is an important constituent of the diet, much of the dietary iron is in the heme-iron form as hemoglobin and myoglobin. Hemeiron is absorbed more efficiently than inorganic iron by the human intestine. Thus, it is important to know how the dose of heme-iron affects iron absorption. The purpose of this study was to establish the dose-effect of heme-iron on the percentage and absolute amount of iron absorbed. Twentyseven healthy women (28- to 50-y-old) were selected to participate in two iron absorption studies. Through the use of iron isotopes (⁵⁹Fe and ⁵⁵Fe), the studies were performed to characterize the dose-response curve of non-heme-iron absorption (ferrous sulfate), and to establish the doseresponse curve of heme-iron absorption (hemoglobin). The labeled hemoglobin was prepared by use of red blood cells from rabbits. The geometric means (±1 SEM range) of nonheme iron absorbed were 0.2 (0.2-0.3), 1.2 (1.0-1.5), 6.7 (5.7-8.0) and 13.0 (11.5-14.6) mg of iron for doses of 0.5, 5, 50 and 100 mg of iron as ferrous sulfate, respectively; and 0.1 (0.1-0.2), 0.4 (0.3-0.4), 2.2 (2.0-2.4) and 2.2 (1.7-3.0) mg of iron for doses of 0.5, 3, 15 and 30 mg of heme-iron as hemoglobin, respectively. The fitted curves for heme and non-heme iron differed (P < 0.04). These results strongly suggest that the heme-iron absorption pathway is saturable. J. Nutr. 133: 2214-2217, 2003.

KEY WORDS: • heme-iron absorption • iron bioavailability iron fortification
iron status

The absorption of dietary iron has been studied extensively with different techniques and under different experimental conditions (1-3). It has been demonstrated that the bioavailability of dietary iron is much more important to iron nutrition than the amount of iron ingested and the composition of meals. One example is the notable difference between the absorption of heme-iron and non-heme-iron (4,5). Hemeiron is principally found in meat as hemoglobin $(Hb)^3$ or myoglobin. This form of iron is easily absorbed because it is not influenced by the many ligands in the diet; furthermore, it is directly taken up into enterocytes by an absorption pathway different from that of non–heme-iron (6,7). Heme-iron is also unaffected by the high pH of the upper small bowel, which renders some forms of inorganic iron insoluble. Both hemeiron and non-heme-iron absorption are influenced by extraluminal factors such as iron stores and rate of erythropoiesis. Absorption is inversely correlated with iron stores and directly correlated with the ervthropoiesis rate (1). It has been demonstrated that an inverse relationship exists between the quantity of non-heme-iron ingested and the percentage of absorption. However, the absolute amount of absorbed non-hemeiron progressively increases with intake (8). This relationship has been clearly demonstrated with non-heme-iron, but it is not known whether this is also the case with heme-iron. The

not known whether this is also the case with heme-iron. The purpose of this study was to establish the dose-effect of heme-iron on the percentage of absorption and absolute absorption of heme-iron. **MATERIALS AND METHODS Subjects.** Twenty-seven healthy women between 28 and 50 y of age were selected to participate in two iron absorption studies (~14 subjects per study). None was pregnant (confirmed by a negative human gonadotrophin chorionic urine test) or lactating, and all were using intrauterine devices as their method of contraception at the time of the study. Previous informed consent was obtained from all the volunteers

Previous informed consent was obtained from all the volunteers হ before this study began. The protocol was approved by the Ethics 9 Committee of the Institute of Nutrition and Food Technology, and the doses of radioactive isotopes used were approved by the Chilean Commission of Nuclear Energy.

Study design. Studies were performed to characterize the dose-Study design. Studies were performed to characterize the dose– response curve of non–heme-iron absorption (ferrous sulfate), and to establish the dose-response curve of heme-iron absorption (Hb).

Isotopic studies. Iron isotopes (⁵⁹Fe and ⁵⁵Fe) of high specific activity were used as tracers (NEN, Life Science Products, Boston, MA). The doses of the compounds labeled with the iron isotopes were given to the subjects in gelatin capsules (number 0; Reutter, Santiago, Chile).

The labeled Hb was prepared by use of red blood cells from rabbits. The New Zealand rabbits, ~3 kg in weight, received an intravenous injection of 74 MBq of ⁵⁵Fe or 37 MBq of ⁵⁹Fe as ferric citrate (NEN, Life Science Products) diluted in 10 mL of 9 g NaCl/L. Fifteen days later, the rabbits were exsanguinated through a cardiac puncture. The radioactive red blood cells were centrifuged (1000 \times g for 15 min at 22°C) and washed in a saline solution, hemolyzed by freezing and finally dehydrated by lyophilization. Labeled freeze-dried red blood cells with a specific activity of 475 kBq of ⁵⁹Fe and 2460 kBq of ⁵⁵Fe per mg of Fe-heme were obtained. These were mixed in dry form with untagged bovine red cells (9), resulting in a dose of 37 kBq of ⁵⁹Fe or 111 kBq of ⁵⁵Fe per 0.5 mg of elemental iron. Elemental iron was

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³ Abbreviations used: FEP, free erythrocyte protoporphyrin; Hb, hemoglobin; Sat, transferrin saturation; SF, serum ferritin.

Iron nutrition status and dose-response of non-heme-iron absorption in women in study A1,2

Subject	Age	Hb	FEP	Sat	SF	Iron bioavailability per dose, mg			
						0.5	5	50	100
	у	g/L	µmol/L RBC	%	μg/L		(
6	34	110	1.56	11.3	4	63.8	63.5	20.9	13.7
8	28	116	2.21	8.3	5	86.5	79.5	26.2	20.3
11	35	128	1.22	19.8	5	37.4	20.3	8.5	16.1
1	37	137	2.02	34.9	5	88.3	46.8	29.0	26.5
12	32	110	1.96	4.7	8	75.7	61.7	24.8	14.2
4	41	142	1.47	39.8	13	72.5	36.0	16.5	9.7
7	31	132	1.72	41.7	13	48.0	30.1	14.7	12.5
5	38	134	1.12	25.4	14	45.3	21.2	13.7	15.5
13	41	126	1.26	33.6	22	24.0	10.6	8.9	9.0
14	47	142	1.36	15.0	28	8.7	4.5	5.5	6.9
9	44	141	1.43	19.5	28	69.2	27.1	23.6	18.5
3	30	147	1.84	46.8	53	77.8	36.0	20.5	21.3
2	50	142	1.31	28.7	58	13.0	7.8	5.7	7.5
10	43	138	1.36	14.0	77	24.7	9.1	4.2	6.5
Mean	38	132	1.56	24.5	15	43.2	24.1	13.4	13.0
SD	7	12	0.34	13.3					
±1 SEM range					12–22	35.5-52.6	19.1-30.4	11.3–16.0	11.5–14.6

¹ Values are means and sp or geometric means and ± 1 SEM ranges.

² Abbreviations: Hb, hemoglobin; FEP, free erythrocyte protoporphiryn; Sat, transferrin saturation; SF, serum ferritin.

determined by atomic absorption spectrometry (Perkin-Elmer Model SIMAA 6100; Perkin Elmer Cetus Instruments, Norwalk, CT). These compounds were packaged in gelatin capsules.

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In both studies, graded iron doses were administered on d 1, 2, 15 and 20, with ⁵⁵Fe being given on d 1 and 15, and ⁵⁹Fe being given on d 2 and 20. The labeled compounds were administered in one capsule containing 0.5 mg of iron labeled with ⁵⁵Fe or ⁵⁹Fe as ferrous sulfate or Hb. At the same time, the graded doses of nonlabeled iron were administered in one or more capsules. The doses were administered after a nocturnal fast, with subjects not being allowed to eat again until 4 h after ingestion of the doses.

In the dose-response study of non-heme-iron (study A) the subjects received 0.5 mg of iron as ferrous sulfate labeled with 111 $kBq^{55}Fe$ or 37 $kBq^{59}Fe$ with 0, 4.5, 49.5 and 99.5 mg of iron as ferrous sulfate. In the dose-response study of heme-iron (study B) the subjects received 0.5 mg of iron as Hb labeled with 111 kBq⁵⁵Fe or 37 kBq ⁵⁹Fe with 0, 2.5, 14.5 and 29.5 mg of iron as Hb. Doses were administered in increasing order.

The graded iron concentrations provided for this study were lower than the first because of the difficulties in providing comparable amounts of iron as Hb (30 mg of heme-iron is equivalent to 75 mL of bovine blood or 10 g of dehydrated blood). Venous blood samples were obtained on d 15 and 35 to measure the circulating radioactivity and to determine the iron status of the subject. Hb, free erythrocyte protoporphyrin (FEP), serum iron, total iron binding capacity, transferrin saturation (Sat) and serum ferritin (SF) were determined in these samples (10).

For the calculation of total radioactivity ingested, aliquots of the compounds were counted in sextuplicate as standards. Measurement of blood radioactivity was performed in duplicate venous samples according to the Eakins and Brown technique (11). The samples were counted within sufficient time to obtain a counting error of <3% in a liquid-scintillation counter (Beckman LS 5000 TD; Beckman Instruments, Fullerton, CA). The percentages of absorption were calculated on the basis of blood volume estimated for height and weight (12), and assuming an 80% incorporation of the radioisotope into erythrocytes (1). This method is reproducible in our laboratory with a CV of 5%.

For purposes of comparison, all the studies currently refer to 100% absorption of the 0.5 mg dose of iron as ferrous sulfate or Hb. Because the percentages of iron absorption and serum ferritin concentrations 1543.224.113.413.02-2235.5-52.619.1-30.411.3-16.011.5-14.6Sat, transferrin saturation; SF, serum ferritin.have a skewed distribution, these values were converted to logarithms before statistical analysis. The results were retransformed into antilogarithms to recover the original units and expressed as geometric means and ± 1 SEM range. The least square mean was used to fit data for the nonlinear dose-response curves $[y = A \cdot x^B \Rightarrow \ln(y) = \ln(A)] + B \ln(x)]$ and to calculate r^2 . Fitted dose-response curves were compared by use of the Mixed Model Analysis the RANDOM STATEMENT in PROC MIXED (SAS Online Doc 8.0, SAS Institute Inc, Cary, NC). Differences were considered significant at P < 0.05.WESUITS

RESULTS

Only 5 of 27 women had iron deficiency anemia (Hb < 120g/L, plus two or more other abnormal iron indicators: Sat < 15%, FEP > 1.42 μ mol/L RBC and SF < 12 μ g/L); 3 had iron deficiency without anemia (Hb > 120 g/L, plus two or more other abnormal iron indicators: Sat < 15%, FEP > 1.42 μ mol/L RBC and SF < 12 μ g/L), and 2 had iron depletion (only SF < 12 μ g/L). Iron status indicators did not differ between the study A and study B groups (Tables 1 and 2).

The geometric means of non-heme-iron bioavailability were 43.2, 24.1, 13.4 and 13.0% for 0.5, 5, 50 and 100 mg doses of iron as ferrous sulfate, respectively; and 27.1, 13.2, 14.6 and 7.4% for 0.5, 3, 15 and 30 mg doses of heme-iron as Hb, respectively. The dose-response curves were relative to 100% absorption of the 0.5 mg dose of iron as ferrous sulfate or Hb (Fig. 1). The ferrous sulfate and Hb curves did not differ when they were analyzed by repeated measures analysis by use of PROC MIXED. However, when iron bioavailability was assessed as the amount of absorbed iron, the fitted curves differed (Fig. 2, repeated measures analysis by use of the PROC MIXED test, P < 0.04). The polynomial regression of the heme-iron absorption curve showed a plateau at 2 mg of iron absorbed when 15 mg of Fe as heme-iron was administered. In comparison, the curve of non-heme-iron absorbed increased to 13 mg of absorbed iron when the subjects received a dose of 100 mg of iron as ferrous sulfate.

TABLE 2

Iron nutrition status and dose-response of heme-iron absorption in women in study B1,2

Subject	Age	Hb	FEP	Sat	SF	Iron bioavailability per dose, mg			
						0.5	3	15	30
	У	g/L	μmol/L RBC	%	μg/L		%		
15	37	102	3.89	9.5	3	41.3	11.5	19.0	13.2
25	43	91	2.97	9.7	5	48.6	19.2	20.8	33.0
16	43	126	1.82	17.8	7	26.7	29.3	15.2	15.2
26	40	131	1.01	22.9	9	30.8	18.6	14.5	11.1
23	42	135	1.82	12.6	11	18.1	13.0	15.5	10.3
18	38	138	0.76	42.3	22	9.4	8.4	8.0	6.8
20	36	140	1.72	31.0	24	17.6	10.8	13.0	2.9
27	44	124	0.81	26.8	25	68.9	10.1	14.2	2.1
22	42	145	2.02	27.3	28	19.5	19.9	17.1	26.7
21	44	141	0.90	26.4	39	32.3	5.9	13.9	0.9
19	44	141	1.31	46.3	45	21.0	14.0	28.1	7.7
24	42	143	1.31	15.9	59	91.5	20.1	11.5	7.8
17	39	144	0.90	34.1	93	10.2	7.9	8.6	4.8
Mean	41	131	1.65	24.8	19	27.1	13.2	14.6	7.4
SD	3	17	0.92	11.7					
±1 SEM range					14–25	22.4-32.7	11.6–15.0	13.3–16.0	5.6-9.8

¹ Values are means and sp or geometric means and ± 1 SEM ranges.

² Abbreviations: Hb, hemoglobin; FEP, free erythrocyte protoporphiryn; Sat, transferring saturation; SF, serum ferritin.

DISCUSSION

The current study presents evidence that heme-iron absorption is a saturable process. On the other hand, non-heme-iron absorption was not saturable within the 0.5 to 100 mg range of Fe tested.

Several studies have provided information on non-hemeiron absorption by enterocytes (13–16). In contrast, there are few studies that report the uptake of heme iron. Heme enters the enterocyte as an intact metalloporphyrin (7). Two mechanisms have been proposed for heme uptake. In a study conducted in rat duodenum, Wyllie and Kaufman (17) showed by electron microscopy that heme enters the enterocyte through some cavities in the apical zone, in a way similar to that which occurs in pinocytosis, an observation also made by Parmley et al. (18). On the other hand, Grasbeck et al. (19) demonstrated the presence of a binding protein of heme in the porcine

14-2522.4-32.711.6-15.013.3-16.05.6-9.8Sat, transferring saturation; SF, serum ferritin.duodenal mucosa that can be the heme receptor on the duo-
denal brush border, suggesting that this receptor could be a
protein of high molecular weight or could be a complex of
various subunits.After the heme enters the enterocyte, it is moved to a
microsomal compartment in the apical zone of the enterocyte
where it is cleaved by a heme-oxygenase-like enzyme to pro-
duce free iron and bilirubin (7). Afterward, the liberated iron
is found in the blood in the form of non-heme-iron (20-22).
The mechanism of the movement of the heme from the
apical-tubule cavity or heme-receptor to the lysosome has yet
to be ombiand. apical-tubule cavity or heme-receptor to the lysosome has yet to be explained.

Scientists have not yet determined the status of the heme after it enters the enterocyte until it arrives at the lysosomes where the absorption of heme-iron may potentially be further limited. Heme-oxygenase and biliverdin reductase are the enzymes required for the catabolism of heme to bilirubin and the release of iron (23), and heme-oxygenase could be increased



FIGURE 1 Dose-response curves of non-heme- and heme-iron absorption in women corrected to 100% absorption of the 0.5 mg dose of iron from ferrous sulfate or dried RBC. Values are means \pm SEM, n = 27. NS, not significant; P > 0.05.



FIGURE 2 Effect of iron dose on absorbed iron in women shown as dose-response fitted curves of non-heme iron and heme iron. Values are means \pm SEM, n = 27.

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by exposure of the cells to heme or metallic ions such as cadmium or cobalt (24). In addition, high levels of hemeoxygenase have been found in iron-deficient enterocytes, suggesting that this enzyme plays an important role in the uptake of heme into the enterocyte (25).

In our study we found that heme-iron absorption was saturable. Heme-iron absorption after ingestion of physiological doses of hemoglobin and myoglobin is limited (26-29); however, the mechanism by which this is produced is not clear. It seems that one of the limiting factors would be the uptake of the heme into the enterocyte, whatever the mechanism. Roberts et al. (30) indirectly demonstrated the presence of a binding protein of heme on the brush border of the rat enterocyte and showed that the capture of heme by the mucosa directly increases the amount of the binding protein on the brush border. They speculated that this binding heme protein may be saturable. If the mechanism of endocytosis proposed by Wyllie and Kaufman (17) is correct, it would be a barrier that limits the intake of heme-iron into the enterocyte.

In summary, our results strongly suggested that the absolute maximum amount of iron absorbed from freeze-dried red blood cells containing over 15 mg of iron as heme is 2 mg, showing that heme-iron absorption is saturable. Further investigation is needed to explain the mechanisms underlying this phenomenon in consumption of actual meals and in subjects of varying iron status. The saturability of heme-iron absorption may be a protective factor to avoid iron overload when iron intake is provided primarily by consumption of meats or blood.

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