# Initial uptake and absorption of nonheme iron and absorption of heme iron in humans are unaffected by the addition of calcium as cheese to a meal with high iron bioavailability[1–3]

*Zamzam K (Fariba) Roughead, Carol A Zito, and Janet R Hunt*

**ABSTRACT**

**Background:** Quantitative data on the mucosal uptake and serosal transfer of nonheme-iron absorption in humans and the effects of calcium on these components are limited.

**Objective:** Our objective was to measure the initial mucosal uptake and the subsequent serosal transfer of nonheme iron and to determine the effects of adding calcium to a meal on both heme- and nonheme-iron retention.

**Design:** Whole-gut lavage and whole-body scintillation counting methods were applied to determine the 8-h uptake of nonheme iron and the 2-wk retention (absorption) of heme and nonheme iron in healthy adults ($n$ = 17) after the consumption of meals of radiolabeled food.

**Results:** The initial uptake and absorption of nonheme iron were 11% and 7%, respectively, and the absorption of heme iron was 15%. Two-thirds of the nonheme iron taken up by the mucosa within 8 h was retained by the body after 2 wk (serosal transfer index: 0.63). Serum ferritin correlated inversely with the initial uptake and absorption of nonheme iron, but not with the nonheme serosal transfer index or the absorption of heme iron. Adding calcium (127 mg in cheese) to the meal did not affect absorption.

**Conclusions:** On the basis of its association with serum ferritin, the initial mucosal uptake was the primary control point for nonheme-iron absorption. An apparent reduction in heme-iron absorption associated with the lavage procedure suggested that uptake of heme iron may take longer and proceed further through the intestine than that of nonheme iron. The absorption of both forms of iron was unaffected by the addition of cheese to this meal with high iron bioavailability. *Am J Clin Nutr* 2002;76:419–25.

**KEY WORDS** Heme iron, nonheme iron, whole-body counting, whole-gut lavage, mucosal uptake, absorption, retention, serosal transfer, humans, calcium, erythrocyte incorporation

## INTRODUCTION

Iron balance is primarily controlled by intestinal absorption (1), but the exact mechanism remains unclear. Current knowledge indicates that the process of iron absorption can be divided into 3 steps. First, soluble iron from the intestinal lumen is taken up by the intestinal mucosal cell at the apical surface, involving specific receptors such as divalent metal transporter-1, stimulator of iron transport, and mobilferrin-integrin for nonheme iron (2, 3), and by yet unidentified receptors for heme iron that enter as an intact metalloporphyrin (4, 5). Second, intracellular processing of the iron may include the release of heme iron from porphyrin by mucosal heme oxygenase (EC 1.14.99.3) (6) and the incorporation of iron into functional or storage compartments such as ferritin (2). Third, iron is either transported to the plasma across the basolateral (serosal) membrane, possibly involving a ceruloplasmin homologue called hephaestin (2), or removed from the body when the epithelial cell is exfoliated (3).

Iron absorption is increased in iron deficiency and in hereditary hemochromatosis (7–10), and the absorptions of both heme and nonheme iron are inversely associated with body iron stores (9, 11–13). Nonheme iron and heme iron are generally believed to enter a common cytosolic pool in the mucosal cells. A common pathway in the final steps of the absorption of heme and nonheme iron was suggested by the observation that nonheme-iron absorption was inhibited by the earlier administration of heme or nonheme iron (14). Orally administered heme iron entered the plasma more slowly than did nonheme iron, but it is not known whether this reflected slower initial mucosal uptake or slower intracellular processing (4, 14, 15).

Investigations of human mucosal uptake and serosal transfer of nonheme iron are limited for nonheme iron and unavailable for heme iron. Early investigators used discriminant analysis of fecal excretion (16, 17) or whole-body retention of a $^{59}$Fe radiotracer and a nonabsorbable radioactive marker (7, 8) to estimate the initial uptake and final retention of nonheme iron. These methods are susceptible to high variability because of individual differences in gastrointestinal transit time and difficulties with the recovery of a fecal marker.

Calcium is the only dietary component known to inhibit the absorption of both heme and nonheme iron (18–20). Although it has been speculated that calcium inhibits the serosal transfer of

[1] From the US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[3] Address reprint requests to ZK Roughead, USDA-ARS, Grand Forks Human Nutrition Research Center, 2420 2nd Avenue North, PO Box 9034, Grand Forks, ND 58202-9034. E-mail: froughea@gfhnrc.ars.usda.gov.

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**TABLE 1**
Characteristics of the participants[1]

| | Value |
|---|---|
| Age (y)[2] | 34 ± 7 [23–54] |
| BMI (kg/m$^2$)[2] | 25.5 ± 3.8 [19.1–34.5] |
| Hemoglobin (g/L)[2] | 145 ± 13 [125–171] |
| Serum ferritin (μg/L)[3] | 53 (25, 110) [11–191] |
| Serum iron (μmol/L)[3] | 11 (8, 16) [7–22] |
| Total-iron-binding capacity (μmol/L)[3] | 52 (47, 59) [43–65] |
| Transferrin saturation (%)[3] | 21.6 (15.0, 30.9) [12.5–50.6] |

[1] $n$ = 9 F and 8 M; range in brackets.
[2] $\bar{x}$ ± SD.
[3] Geometric $\bar{x}$ (−SD, +SD).

iron rather than its mucosal uptake (19), such an action has not been measured.

The specific objectives of this study were *1*) to develop a method using whole-gut lavage and whole-body counting procedures to compare the initial uptake and serosal transfer components of nonheme-iron absorption, *2*) to determine the relation of serum ferritin to these components of nonheme-iron absorption, and *3*) to determine the effect of adding calcium on the initial uptake of nonheme iron and the absorption of both heme and nonheme iron.

## SUBJECTS AND METHODS

### Subjects

The study participants were 9 women and 8 men with normal hemoglobin and a range of iron stores (serum ferritin: 11–191 μg/L) (**Table 1**). Participants were recruited through public advertising and selected after an interview and blood analysis to establish that they were ≥21 y of age, had no apparent underlying disease, had a normal hemoglobin concentration (≥12 g/L for women, ≥14 g/L for men), had a serum ferritin concentration <450 μg/L, had not donated blood for 2 y, and had not used iron supplements for ≥6 mo.

The participants agreed to discontinue all nutritional supplements when their applications were submitted, generally 6–12 wk before the beginning of the study. None of the participants routinely used medications. Women were excluded if they had been pregnant within the past year or were currently breast-feeding.

The participants gave informed consent. The study was approved by the University of North Dakota's Radioactive Drug Research Committee and Institutional Review Board and by the US Department of Agriculture's Human Studies Review and Radiological Safety committees.

### General protocol

Gastrointestinal lavage and whole-body scintillation counting procedures were applied to distinguish between the initial mucosal uptake and the subsequent serosal transfer and retention of nonheme iron. Healthy volunteers consumed a test meal labeled with $^{59}FeCl_3$ and [$^{55}$Fe]heme radiotracers. Each volunteer participated twice, at intervals of 4 wk, with cheese added to 1 of the 2 meals in random order. Each time, the entire gut was cleansed 8 h later with an orally administered lavage solution. The initial uptake of nonheme iron was determined by comparing the amount of $^{59}$Fe in the body immediately after the lavage procedure with the initial dose; both were measured by whole-body scintillation counting. The retentions of nonheme and heme iron were determined 2 wk after the test meal by whole-body scintillation counting and by isotopic analyses of blood. A serosal transfer index for $^{59}$Fe was calculated as the percentage of the administered dose retained after 2 wk divided by that retained after 8 h; thus, a serosal transfer index expresses serosal transfer as a fraction of mucosal uptake. The terms absorption and whole-body retention are used interchangeably, and they refer to the retention of the isotopes in the body 2 wk after the administration of the test meal, expressed as a fraction of the initial dose.

### Test meal

The test meal, generally patterned after that published by Lynch et al (9), consisted of ground beef (90 g), a hamburger bun (53 g), French fries (68 g), apple juice (240 g), and tomato ketchup (40 g) and contained 1.1 mg heme Fe and 4.3 mg total Fe by analysis. To test for the effects of calcium, 28 g cheese (127 mg Ca by analysis) was added in random order to 1 of 2 test meals consumed by participants at 4-wk intervals. The radiotracers $^{55}$Fe (19 kBq as rabbit hemoglobin) and $^{59}$Fe (37 kBq as $FeCl_3$) and 0.3 mg of a fecal marker, dysprosium (as $DyCl_3 \cdot 6H_2O$; Sigma, St Louis), were added to the cooked hamburger patty, which was briefly reheated in a microwave oven before being served. Dose aliquots for each isotope were prepared in 0.5% EDTA when the test meals were labeled. The test meals were weighed to 1% accuracy and consumed quantitatively (with the aid of spatulas and rinse bottles) at the research center. The participants fasted for ≥10 h before and 8 h after the test meal. A carbonated, caffeine-free, sugar-containing beverage was allowed at the midpoint of the 8-h fast to alleviate any discomfort due to fasting. Water was allowed ad libitum.

The $^{59}$Fe and $^{55}$Fe isotopes were purchased from NEN Life Science Products (Boston). Radiolabeled hemoglobin was obtained by intravenously injecting 74 MBq (2 mCi) $^{55}$Fe into an iron-deficient, pathogen-free rabbit; exsanguinating the animal 2 wk later; and removing the stroma by lysing and centrifugation (21). The specific activity of the final preparation was 0.585 kBq/μg Fe. The amounts of iron added to each test meal as a result of labeling with $^{55}$Fe and $^{59}$Fe were ≤32 μg and ≤0.4 μg, respectively.

### Initial mucosal uptake of nonheme iron

After each test meal and the subsequent 8-h fast, the participants were admitted to a private room in the metabolic ward of the Grand Forks Human Nutrition Research Center for the whole-gut lavage procedure. The participants drank 4 L of an isosmotic-isotonic polyethylene glycol solution (240 mL every 10 min; GoLYTELY, Braintree Laboratories, Inc, Braintree, MA).

The initial uptake of nonheme iron was determined as the fraction of the isotope remaining in the body, immediately after the completion of the whole-gut lavage procedure, relative to the total amount consumed in the test meal, both determined with the use of a whole-body scintillation counter. This custom-made whole-body counter, described elsewhere (22), detects the γ-emitting $^{59}$Fe radioisotope (but not the weak X-ray emissions of $^{55}$Fe) by using 32 crystal NaI(T1) detectors (10 × 10 × 41 cm each) arranged in 2 planes above and below a bed. The initial whole-body activity from the test meal was determined from the whole-body activity measured 1–3 h after the test meal (before any unabsorbed isotope was excreted).

The amount of fecal marker added to the test meal in this study (0.3 mg Dy) was insufficient for reliable detection of dysprosium.

The use of a fecal marker was not necessary for the determination of the mucosal uptake measurements for nonheme iron, however, because $^{59}Fe$ retention was measured by whole-body counting. (The determination of the mucosal uptake of heme iron from stool measurements of $^{55}Fe$ and $^{59}Fe$ will require further development of the method and is not reported here.)

### Measurements of heme- and nonheme-iron absorption and erythrocyte incorporation

Nonheme-iron absorption was determined by whole-body scintillation counting as the portion of initial whole-body activity that remained after 2 wk (day 15), with correction for physical decay and for background activity measured a few days before the meals. In a previous study, the slopes of semilogarithmic whole-body retention plots for 4 wk after isotope administration were not consistently different from zero, which indicated that iron excretion was minimal and that it was unnecessary to correct for endogenous excretion of iron during the 2 wk after isotope administration (23).

Radioisotope concentrations in blood were measured after 2 wk (day 15), as described by Bothwell et al (24) but using Ultima Gold AB liquid scintillation cocktail (Packard Instrument Co, Meriden, CT), and were expressed as fractions of the administered radioisotope, measured from dose aliquots prepared when the hamburgers were labeled. Although the isotope recovery from digested blood samples was not determined directly, it was indirectly corrected for by expression of the sample results as a fraction of a dose aliquot that was concurrently digested with nonradiolabeled blood. The blood retention of $^{59}Fe$, expressed as a percentage of the administered dose, was determined from the blood radioisotope concentration together with an estimate of total blood volume based on body height and weight (25, 26). The erythrocyte incorporation of iron, expressed as a percentage of the absorbed nonheme iron, was determined by dividing the fractional blood retention of $^{59}Fe$ by the fractional absorption of $^{59}Fe$ as measured by whole-body counting. Heme-iron absorption was determined by multiplying the nonheme-iron absorption (determined by whole-body counting) by the ratio of $^{55}Fe$ to $^{59}Fe$ in the blood, with correction for radioactive decay and for background activity measured before the test meal, and assuming similar erythrocyte incorporation of absorbed heme and nonheme iron.

When heme- and nonheme-iron absorption measured by using whole-body scintillation counting or blood isotope analyses (and assuming 80% incorporation of the newly absorbed isotope into blood) were compared, the 2 independent methods were highly and significantly correlated in this study ($r = 0.94$ and 0.99 for heme and nonheme iron, respectively; $P < 0.0001$) and in previous studies (13, 27). However, as reported earlier (13), the iron-absorption measurements obtained by using only the blood analyses, as compared with those obtained by whole-body counting, slightly exaggerated the strength of the relation between iron absorption and serum ferritin, especially for heme iron, because serum ferritin was also inversely correlated with the erythrocyte incorporation of absorbed iron.

### Diet analysis

Duplicate aliquots of the test meals were digested with concentrated nitric acid and 70% perchloric acid by method (II)A of the Analytic Methods Committee (28). The iron content of the digestates was determined by inductively coupled argon plasma emission spectrophotometry. Analytic accuracy was monitored through periodic analyses of certified standard reference materials from the National Institute of Standards and Technology. The measurements were 95 ± 9% ($\bar{x}$ ± SD) of certified values for iron.

The same digestion and inductively coupled argon plasma emission spectrophotometry methods were used to measure nonheme iron in the test meal, after extraction (29). Heme iron in the test meal was calculated as the difference between total and nonheme iron. Our previous analyses indicated that the cooking procedures (baking and briefly reheating in the microwave) had negligible effects on the heme iron content of beef.

### Other analyses

Hemoglobin and hematocrit were measured with a Celldyne 3500 System (Abbott Laboratories, Abbott Park, IL). Serum iron was measured colorimetrically by using a Cobas Fara Chemistry Analyzer (Hoffmann-La Roche, Inc, Nutley, NJ) with a commercial chromagen (Ferene; Raichem Division of Hemagen Diagnostics, San Diego). Iron-binding capacity was similarly determined after the addition of a known amount of ferrous iron to the serum sample under alkaline conditions. Percentage transferrin saturation was calculated from serum iron and total-iron-binding capacity. C-reactive protein was measured by nephelometry (Behring Diagnostics Inc, Westwood, MA) to help detect increases in serum ferritin related to inflammation. The C-reactive protein measurements were within normal limits for all volunteers.

### Statistics

Data on the initial mucosal uptake and retention of heme and nonheme iron, serum iron, total-iron-binding capacity, transferrin saturation, and serum ferritin were logarithmically transformed, and geometric means are reported. Differences between the initial mucosal uptake and retention of heme and those of nonheme iron were determined by analysis of variance (30). Simple linear regression analyses (30) were used to assess additional relations between transformed variables. The between- and within-individual variability estimates were calculated by using an analysis of variance model and are expressed as CV (**Table 2**).

## RESULTS

### Effect of calcium

The addition of a modest 127 mg Ca as cheese to this meal with a relatively high bioavailability of iron was insufficient to significantly affect heme- or nonheme-iron absorption (Table 2). Therefore, the results for the 2 meals (with and without cheese) were averaged for each participant for all further data analyses (**Table 3**). The degree of variability of the absorption measurements between individuals and within each individual was greater for nonheme iron than for heme iron (Table 2).

### Initial mucosal uptake of nonheme iron and 2-wk retention (absorption) of heme and nonheme iron

The initial mucosal uptake of nonheme iron, measured 8 h after the test meal and expressed as a percentage of the dose (geometric $\bar{x}$: −SD, +SD), was 11.1% (6.8, 18.2), and the 2-wk retention was 7% (3.4, 14.4) (Table 3). Thus, the transfer of nonheme iron to the bloodstream was incomplete and resulted in a serosal transfer index of 0.63 (0.44, 0.91). This index represents the amount of isotope transferred to the body as a proportion of that initially

**TABLE 2**

Initial uptake and serosal transfer index of nonheme iron, absorption of heme and nonheme iron, and erythrocyte incorporation of absorbed iron in healthy participants who ate hamburger and cheeseburger test meals[1]

| Subject sex and age (y) and meal sequence | Serum ferritin | | Nonheme iron: Uptake | | Nonheme iron: Absorption | | Nonheme iron: Serosal transfer index | | Heme-iron absorption | | Erythrocyte incorporation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H | C | H | C | H | C | H | C | H | C | H | C |
| | *μg/L* | | %[2] | | %[2] | | | | %[2] | | %[2] | |
| F, 52; H-C | 26 | 22 | 23.5 | 26.3 | 13.6 | 22.2 | 0.58 | 0.85 | 17.1 | 16.7 | 84 | 81 |
| F, 40; C-H | 27 | 25 | 15.4 | 16.8 | 13.9 | 13.3 | 0.90 | 0.79 | 19.9 | 21.4 | 83 | 78 |
| F, 41; C-H | 6 | 9 | 27.7 | 27.6 | 25.3 | 23.8 | 0.91 | 0.86 | 23.2 | 25.8 | 84 | 88 |
| F, 29; C-H | 76 | 54 | 6.4 | 10.4 | 2.3 | 6.2 | 0.36 | 0.60 | 8.4 | 10.8 | 56 | 58 |
| M, 31; C-H | 91 | 89 | 9.3 | 6.1 | 5.9 | 3.9 | 0.64 | 0.64 | 28.0 | 17.6 | 64 | 67 |
| F, 37; H-C | 80 | 120 | 9.5 | 7.1 | 7.3 | 5.1 | 0.77 | 0.73 | 24.0 | 18.0 | 80 | 65 |
| M, 39; H-C | 143 | 137 | 7.3 | 3.7 | 2.5 | 1.7 | 0.35 | 0.46 | 13.7 | 10.0 | 61 | 64 |
| M, 34; C-H | 87 | 86 | 4.3 | 5.9 | 1.9 | 3.2 | 0.45 | 0.54 | 12.6 | 13.1 | 57 | 56 |
| M, 30; H-C | 83 | 76 | 7.1 | 18.7 | 4.2 | 12.8 | 0.59 | 0.68 | 6.7 | 19.5 | 68 | 69 |
| M, 23; H-C | 67 | 64 | 8.9 | 9.3 | 6.3 | 6.8 | 0.70 | 0.73 | 11.8 | 14.9 | 68 | 60 |
| F, 37; H-C | 64 | 52 | 15.6 | 20.8 | 13.8 | 18.9 | 0.89 | 0.91 | 10.1 | 14.5 | 73 | 78 |
| F, 34; H-C | 25 | 23 | 19.1 | 15.7 | 17.0 | 9.2 | 0.89 | 0.59 | 16.2 | 16.4 | 78 | 77 |
| M, 35; C-H | 201 | 203 | 13.9 | 15.6 | 8.1 | 10.5 | 0.58 | 0.67 | 18.2 | 28.7 | 61 | 60 |
| F, 27; C-H | 34 | 32 | 9.8 | 12.0 | 8.7 | 11.7 | 0.89 | 0.98 | 20.3 | 23.1 | 81 | 87 |
| M, 24; H-C | 48 | 48 | 6.6 | 14.5 | 4.6 | 7.1 | 0.70 | 0.81 | 8.7 | 18.1 | 63 | 72 |
| F, 33; H-C | 31 | 24 | 8.2 | 16.5 | 2.2 | 2.8 | 0.27 | 0.17 | 8.5 | 10.2 | 79 | 84 |
| M, 31; H-C | 21 | 34 | 11.2 | 7.4 | 8.8 | 3.5 | 0.79 | 0.48 | 12.8 | 10.8 | 85 | 92 |
| Mean[3] | 54 | 55 | 10.6 | 11.6 | 6.6 | 7.4 | 0.62 | 0.63 | 14.5 | 16.2 | 71 | 72 |
| −SD | 21 | 22 | 6.4 | 6.6 | 3.0 | 3.4 | 0.43 | 0.42 | 9.7 | 11.8 | 62 | 61 |
| +SD | 143 | 138 | 17.5 | 20.6 | 14.4 | 16.1 | 0.91 | 0.95 | 21.7 | 22.4 | 84 | 83 |
| $CV_{within}$ (%)[4] | 5.0 | | 12.3 | | 19.6 | | 16.4 | | 9.0 | | 1.3 | |
| $CV_{between}$ (%)[4] | 23.6 | | 18.6 | | 34.5 | | 25.5 | | 9.9 | | 3.3 | |

[1] H, hamburger test meal; C, cheeseburger test meal. None of the iron-absorption values were affected by the test meal ($P > 0.05$); therefore, the data were pooled for further statistical analyses.

[2] Data are expressed as a percentage of the dose, except for erythrocyte incorporation, which is expressed as a percentage of the absorbed dose.

[3] Except for the serosal transfer index, values are geometric means ($n = 17$).

[4] Calculations based on log-transformed data.

taken up by the mucosal cell. Thus, relative to the amount initially taken up by the mucosal cell (0.35 mg), about two-thirds of the nonheme iron (0.22 mg) was transferred to the serosal side (ie, absorbed), as indicated by whole-body retention after 2 wk. The higher bioavailability of heme iron was evident by its higher fractional absorption (15.4%; 11.2, 21.2), expressed as a percentage of the dose remaining in the body 2 wk later, which was significantly greater than that for nonheme iron (15% compared with 7%; $P < 0.001$; Table 3). In absolute amounts, more nonheme (0.22 mg; 0.11, 0.45) than heme (0.15 mg; 0.11, 0.21) iron was absorbed from the meal ($P < 0.02$).

To assess whether the lavage procedure affected the efficiency of iron absorption, the absorption values observed in this study were compared with those from an earlier study (13) that used a slightly different hamburger test meal that also had a high iron bioavailability. Serum ferritin significantly predicted heme- and nonheme-iron absorption by regression analysis in that study (respectively: $r = -0.45$, $P < 0.001$; $r = -0.66$, $P < 0.0001$). The nonheme-iron retention in this study was similar (geometric mean; −SD, +SD) (7%; 3, 14) to that predicted by the regression model (6%; 4, 9; $P = 0.14$), which suggests that the bulk of the initial uptake for nonheme iron had already taken place in the proximal intestine (in the allotted 8 h) and that the lavage procedure did not interfere with nonheme-iron absorption. However, the heme-iron absorption in the present study (geometric $\bar{x}$: −SD, +SD) (15%; 11, 21) was lower than that predicted from the regression model of the earlier study (25%; 21, 29; $P < 0.0001$) (13). This suggests the possibility that heme iron uptake may continue beyond the proximal small intestine and that the lavage procedure truncated the heme iron-absorption process.

**TABLE 3**

Correlation of iron-absorption variables with serum ferritin[1]

| | *r* | *P* |
|---|---|---|
| Nonheme iron | | |
| Initial uptake (% of dose) | −0.64 | 0.006 |
| Absorption/2-wk retention (% of dose) | −0.54 | 0.024 |
| Serosal transfer index (% of mucosal uptake) | −0.35 | NS |
| Erythrocyte incorporation (% of absorbed dose) | −0.79 | 0.0001 |
| Heme iron | | |
| Absorption/2-wk retention (% of dose) | −0.15 | NS |

[1] Data were logarithmically transformed ($n = 17$).

### Correlations of serum ferritin with initial uptake, serosal transfer index of nonheme iron, absorption of heme and nonheme iron, and erythrocyte incorporation of iron

The initial mucosal uptake of nonheme iron correlated inversely with serum ferritin (ln transformed data: $r = -0.64$, $P < 0.01$; Table 3; **Figure 1**). The absorption or whole-body retention of nonheme iron also correlated inversely with serum ferritin, but this association was weaker (ln transformed data: $r = -0.54$, $P < 0.05$; **Figure 2**). The serosal transfer index of nonheme iron did not correlate with serum ferritin (Table 3). The absorption of heme iron did not correlate with iron status, as indicated by serum ferritin

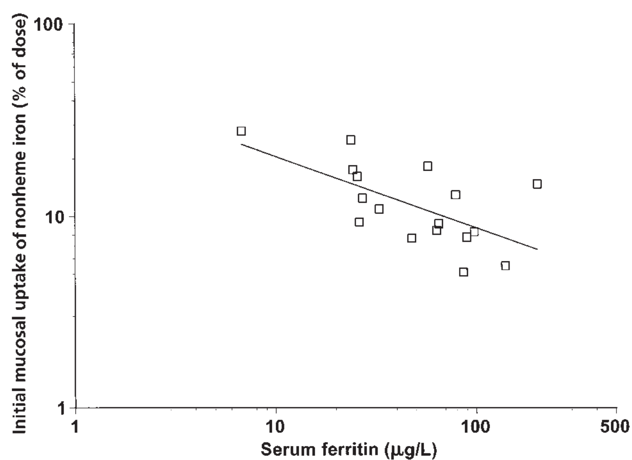



**FIGURE 1.** Inverse correlation between the initial mucosal uptake of nonheme iron and serum ferritin ($r = -0.64$, $P < 0.01$). Data are shown on a logarithmic scale. $n = 17$.

(Figure 2, Table 3). This remained true whether heme-iron absorption was determined by whole-body counting or by using only blood isotope measurements (with the common assumption of 80% erythrocyte incorporation of absorbed iron).

Two weeks after the test meal, 72% of the newly absorbed nonheme iron was incorporated into the blood (Table 2). This erythrocyte incorporation correlated inversely with serum ferritin (ln transformed data: $r = -0.79$, $P < 0.001$) (Table 3). Serum transferrin saturation did not correlate with any of the heme- and nonheme-iron absorption components.

## DISCUSSION

In this study, whole-gut lavage and whole-body scintillation counting methods were combined to determine the initial uptake of nonheme iron and the absorption of both heme and nonheme iron from a test meal ingested by healthy individuals.

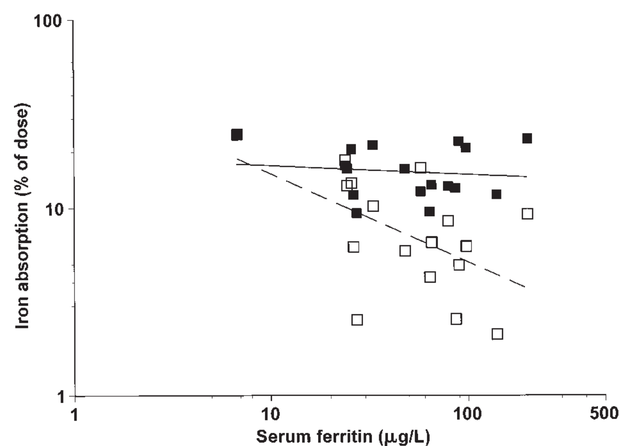



**FIGURE 2.** Correlation between the absorption of heme (■) and nonheme (□) iron and serum ferritin. Serum ferritin correlated inversely with nonheme iron ($r = -0.54$, $P < 0.03$), but not with heme iron ($r = -0.15$, NS). Data are shown on a logarithmic scale. $n = 17$.

Although previous investigators estimated the initial mucosal uptake and serosal transfer of nonheme iron from a test solution given with (7) or without (8, 16, 17) a meal, these measurements have not been reported with the sensitivity afforded by using a combination of whole-gut lavage and whole-body scintillation counting.

The initial uptake of iron can be measured accurately only shortly after the ingestion of the test dose and before a significant number of mucosal cells die and are exfoliated into the lumen of the intestine. Initial uptake measurements are further complicated by the large amount of unabsorbed iron present in the intestinal lumen. The participants in one study (19) were given a laxative 6 h after the consumption of the test meal to ensure a timely bowel movement (31). The approach described in the current study eliminates the variability caused by differences in gastric transit time. The choice of an 8-h period for initial uptake was arbitrary, allowing substantial time for passage through the upper intestinal tract (32) but insufficient time for considerable sloughing of mucosal cells and also minimizing the contamination by the luminal exogenous iron with iron from the sloughed mucosal cells (life span 2–3 d) (33). The complete purging of the intestinal contents coupled with the sensitivity of the whole-body counter provides an accurate estimation of the mucosal uptake after the allotted time (in this case, 8 h), without relying on a fecal marker.

The initial mucosal uptake of nonheme iron (geometric $\bar{x}$: −SD, +SD) of 11% (7, 18) in the current study agrees closely with the 12 ± 5% ($\bar{x}$ ± SD) reported in another study of persons with normal iron status (7). In the current study, the amount of nonheme iron retained by the body was ≈63% of mucosal uptake, or 7% of ingested iron, which was somewhat higher than the 31% of mucosal uptake, or 4% of ingested iron, reported by Powell et al (7). When iron absorption was measured from a test solution rather than from a meal (8), the initial mucosal uptake of nonheme iron ($\bar{x}$ ± SD: 38% ± 17) was greater (3), as expected, with ≈54% fractional serosal transfer and 20 ± 11% retention of ingested iron. These differences in uptake and retention of iron may reflect differences in the bioavailability of iron in the test meal or test solution, the iron status of the participants, or differences in the methods.

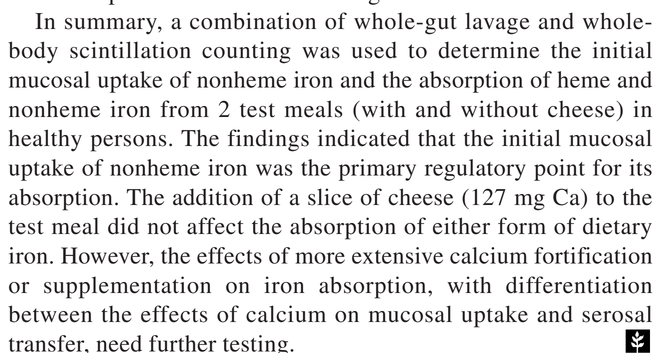
In the current study, initial mucosal uptake and subsequent absorption of nonheme iron both were inversely correlated with serum ferritin (Figures 1 and 2). This agrees with the results of a previous study (7) in which the mucosal uptake and absorption of nonheme iron were significantly higher in persons with iron deficiency anemia (34% and 30%, respectively) than in those with normal iron status (12% and 4%, respectively). Those investigators reported that the fractional transfer of iron was also greater in persons with anemia than in healthy persons (nearly 100% compared with 31 ± 21%) (7). Similarly, in another study, the mucosal uptake, serosal transfer, and retention of nonheme iron were greater in patients with iron deficiency than in healthy participants (8). Serum ferritin correlated inversely with the initial uptake of nonheme iron, but not with the serosal transfer index, which is consistent with the notion that the initial uptake into the intestinal cell, rather than its subsequent serosal transfer, is the primary control point for nonheme-iron absorption (Table 3; Figures 1 and 2). In another study, which used kinetic modeling with dual isotopes, it was found that mucosal uptake was the rate-limiting step in nonheme-iron absorption in normal human participants and that it was inversely related to body iron stores, as measured by serum ferritin (34).

A similar inverse correlation between heme-iron absorption and serum ferritin was not evident in the current study (Figure 2; Table 3). However, a significant inverse relation was reported in previous studies by this laboratory (13) and others (9, 11, 12, 35, 36), showing some degree of biological control that is less than that observed for nonheme iron. It has been shown that the whole-gut lavage procedure, administered after consumption of a test meal (≈12 h), does not alter the efficiency of intestinal absorption of calcium, electrolytes, or sugars (37), but, in the current study, heme-iron absorption was less than predicted. This suggests that heme-iron absorption may continue beyond the proximal intestine and that it might have been truncated by the lavage procedure. Thus, the absence of a significant correlation between iron status and heme-iron absorption in this study may be due to incomplete heme-iron absorption. Truncated absorption may also account for the lower variability for heme-iron absorption than for nonheme-iron absorption (Table 2).

Nonheme-iron absorption did not appear to be affected by the lavage procedure in this study. In animal studies, nonheme-iron absorption has been reported to be either unaffected by (38) or inversely related (39) to intestinal motility.

Calcium has been shown to inhibit both heme- and nonheme-iron absorption, possibly by competing in a final transfer step within the enterocyte (11). This inhibition depends on the calcium dose as well as the iron bioavailability of the meal (18, 19, 40, 41). The results of the current study indicate that the addition of a modest amount of calcium (127 mg) to a meal with high iron bioavailability does not affect the initial uptake of nonheme iron or the absorption of either heme or nonheme forms of dietary iron. Further development of the present method will allow testing of the effect of more extensive calcium fortification of a meal on the initial uptake of heme and nonheme iron, as compared with their long-term retention.

In the current study, a large portion of the nonheme iron taken up by the mucosal cell (37%) was not transferred to the body. This indicates that, although the uptake of iron by the mucosal cell was the primary control point for the regulation of iron absorption (nonheme), serosal transfer also played an important role. It has been proposed that mucosal ferritin may control iron absorption by blocking serosal transfer. This "ferritin block" hypothesis (42) is supported by the findings of lower duodenal ferritin messenger RNA in persons with iron deficiency, higher concentrations in persons with secondary iron overload (43), direct correlation of mucosal ferritin to iron stores, and an inverse correlation of mucosal ferritin with iron absorption (44). Furthermore, fecal ferritin (an indicator of mucosal ferritin content) responded to changes in dietary iron bioavailability (23, 27) and intake (13, 45). The relative importance of mucosal ferritin in the regulation of iron absorption needs further investigation.



In summary, a combination of whole-gut lavage and whole-body scintillation counting was used to determine the initial mucosal uptake of nonheme iron and the absorption of heme and nonheme iron from 2 test meals (with and without cheese) in healthy persons. The findings indicated that the initial mucosal uptake of nonheme iron was the primary regulatory point for its absorption. The addition of a slice of cheese (127 mg Ca) to the test meal did not affect the absorption of either form of dietary iron. However, the effects of more extensive calcium fortification or supplementation on iron absorption, with differentiation between the effects of calcium on mucosal uptake and serosal transfer, need further testing.

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