

Evidence that iron stores regulate iron absorption— a setpoint theory¹⁻³

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ABSTRACT To evaluate the concept of a predetermined "setpoint" for iron stores, 20 healthy elderly individuals (12 blood donors and 8 nondonors) were studied to examine the effects of changes in iron stores on iron absorption. Oral iron-absorption tests revealed a statistically significant increase ($P < 0.001$) in iron absorption in donors, $7.4 \pm 3.6\%$ ($\bar{x} \pm SD$) compared with $2.5 \pm 1.4\%$ in nondonors. In a comparison of percent iron absorption with changes in baseline iron stores, a statistically significant correlation was noted ($r^2 = 0.702$, $P < 0.001$). Thus, reductions in iron stores were correlated with increases in iron absorption. Basal gastric acid output was found to be within normal limits (1.62–10.20 mmol/h) in all elderly subjects and unrelated to iron stores. These findings are consistent with iron absorption being regulated according to the degree of depletion of iron stores from predonation values and is consistent with a proposed setpoint theory of iron stores. *Am J Clin Nutr* 1994;59:1376–80

KEY WORDS Iron stores, blood donations, iron absorption, blood donors, gastric acid, elderly men and women

Introduction

Iron absorption is known to be a complex process influenced by many factors, including presence or absence of iron deficiency, rate of erythropoiesis, bioavailability of dietary iron, and gastric acidity. Body iron stores clearly affect intestinal mucosal iron absorption. Recent studies by Cook et al (1) demonstrate that an individual's iron stores, rather than dietary bioavailability of iron, is the principle determinant of iron absorption. In brief, an adaptive response occurs that regulates mucosal iron absorption according to one's iron stores, increasing absorption when iron stores are depleted and reducing absorption as iron stores are repleted (2). The mechanism by which the intestinal mucosal cell controls iron absorption has not been clearly delineated (3).

In a recent study of elderly blood donors by Garry et al (4), an adaptive response in iron absorption was observed as donor iron stores were progressively lowered by scheduled blood donations. A second important observation was that iron stores did not change significantly over 2 y in a control group of elderly nondonors (4), suggesting steady-state iron stores. Yet elderly nondonors who entered the study with very different iron stores (5 vs 15 mg/kg) did not show any individual changes in iron stores over 2 y on similar oral intakes of iron. From these observations, the authors proposed the existence of a theoretical "setpoint" for iron stores, which varied from subject to subject, as

an explanation of the between-subject differences in baseline iron stores. Furthermore, they proposed that differences in adaptive responses in iron absorption between individuals might be due to differences in the extent of deviation of iron stores from the theoretical setpoint in each individual. The extent of upregulation of iron absorption as iron stores fall would therefore depend on the individual's setpoint for iron stores. This does not exclude the possibility that other factors known to affect iron absorption may also contribute to the wide variation in baseline iron stores observed in this elderly cohort. For example, it is known that gastric acid is an important intraluminal factor necessary for optimal nonheme iron absorption (5). In the total elderly population, in which up to 30% of subjects have atrophic gastritis and associated hypochlorhydria (6), an effect of impaired gastric acid secretion on iron absorption could contribute to differences in individual iron stores. Because of this possibility, in the present group of elderly subjects we measured iron absorption, iron stores, and basal gastric acid output (BAO) and examined the possibility that between-subject differences in iron stores might be due to differences in BAO.

Subjects and methods

Subjects

Study subjects were chosen from among participants in a study of the effects of blood donation in elderly individuals. A detailed description of these subjects and the study has been published (7). In brief, 244 men and women were randomly assigned to donor and nondonor (control) groups. The donor group consisted of 110 individuals (58 men, 52 women) who donated 1 unit of blood (≈ 485 mL) every 8–10 wk. The control group (nondonors) was seen at the same frequency as were the donors but did not donate blood. A blood sample (≈ 7 mL) was obtained from both donors and nondonors at each visit for the tests listed below.

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For the present study, a letter was mailed to all subjects inviting their participation. The subjects who replied were interviewed by one of the authors (MWG) and those with a history of dyspepsia, those who use antiulcer drugs, those with upper gastrointestinal disease, or those who had had abdominal surgery (except appendectomy) were excluded. After screening, 13 participants from the donor group (9 women, 4 men) and 8 participants from the nondonor group (4 women, 4 men) were eligible for inclusion in the present study.

The study was approved by the Human Research Review Committee of the University of New Mexico School of Medicine. Informed consent was obtained from each participant.

Laboratory measurements

Hemoglobin (Hb) was determined by the cyanomethemoglobin method. Serum iron concentration and total-iron-binding capacity (TIBC) were measured by an automated colorimetric method with sulfonated bathophenanthroline as the chromogen (8). Transferrin saturation was calculated by expressing serum iron as a percent of the TIBC. Serum ferritin determinations were performed by using a two-site enzyme immunoassay (Ferrizyme Kit; Abbott Laboratories, Chicago).

Serum iron, ferritin, and TIBC were checked repeatedly for long-term reproducibility by using a lyophilized human serum control (Lyphocheck; Bio Rad Laboratories, Anaheim, CA). For serum controls with known iron concentrations of 19.2 $\mu\text{mol/L}$ (107 $\mu\text{g/dL}$) and 40.6 $\mu\text{mol/L}$ (227 $\mu\text{g/dL}$) CV for iron measurements over 1 y were 3.51% ($n = 315$) and 2.5% ($n = 276$), respectively. The CVs for TIBC measurements over a 1 y were 4.8% ($n = 367$) and 5.4% ($n = 335$) for serum concentrations of 40.9 $\mu\text{mol/L}$ (228 $\mu\text{g/dL}$) and 54.4 $\mu\text{mol/L}$ (304 $\mu\text{g/dL}$), respectively. The CVs for ferritin determinations over 6 mo were 6.3% ($n = 78$), 7.0% ($n = 77$), and 7.4% ($n = 81$) for serum concentrations of 275, 160, and 20 $\mu\text{g/L}$, respectively. Hb determinations were monitored by using a commercial hemoglobin control solution (Diff-Trol; Dade Co, Miami). The CV for Hb determinations was 1.53% ($n = 248$) over 1 y, when a control with an Hb concentration of 140 g/L was used.

Basal gastric acid output

BAO was measured as follows. After an overnight fast, a 16-F nasogastric tube was passed through the nose and positioned by using the water-recovery test (9). After discarding residual gastric contents, gastric juice was collected by manual aspiration with a 50-mL syringe, in 15-min aliquots. Aliquot volume was recorded to the nearest milliliter. With a pH meter (model 610A; Fisher Scientific Co, Springfield, NJ) calibrated daily with reference buffer solutions of pH 7.00, 4.01, and 1.00, total titratable acidity was determined by titrating each total sample against 0.1 mol NaOH/L to pH 7.0. BAO was defined as the sum of four 15-min collections titrated separately, and was expressed as mmol/h.

Iron absorption

A standard oral iron-absorption test (10, 11) was performed on each participant. After an overnight fast, at 0800, a baseline blood sample (7 mL) was drawn for serum iron, TIBC, ferritin, hematocrit, and Hb measurements. An oral dose of 66 mg (10 mL) ferrous fumarate elixir (Forest Pharmaceuticals, Inc, St Louis) was given followed by 240 mL water. Participants remained fast-

ing for 3 h, at which time another blood sample was obtained for serum iron and TIBC measurements.

Values for iron absorption were calculated by the following methods. The predicted blood volume (PBV) was calculated for each subject according to the following equations (12):

$$\begin{aligned} \text{Men: PBV(L)} &= 0.3669(H^3) + 0.03219(W) + 0.6041 \\ \text{Women: PBV(L)} &= 0.3561(H^3) + 0.03308(W) + 0.1833 \end{aligned}$$

where, H is height (m) and W is body mass (kg).

The predicted plasma volume (PPV) was then calculated according to the following equation:

$$\text{PPV(L)} = 100\% - \text{corrected hematocrit(\%)} \times \text{PBV}$$

Each subject's laboratory-determined hematocrit was corrected for the "trapped plasma" in centrifuge hematocrit measurement by using a correction factor of 0.96 (12). The differences in the 0- and 3-h serum iron concentrations (Δ serum iron in $\mu\text{mol/L}$) were corrected for diurnal variation by subtracting 7 $\mu\text{g/dL}$ from the 3-h change as determined by Seligman et al (11). The amount of ingested iron absorbed was calculated according to the following equation:

$$\text{Iron absorbed(mg)} = \text{corrected } \Delta \text{ 3-h serum iron } (\mu\text{mol/L}) \times 0.05585 \times \text{PPV(L)}$$

The amount of iron absorbed was also expressed as a percent of the standard oral dose (66 mg) of iron.

Estimates of body iron

Body iron was divided into a storage compartment and a functional compartment that consisted of circulating Hb and all non-storage tissue iron, according to the procedure of Cook et al (13). Positive values of iron stores represent the amount of iron that could be removed from the body without inducing a deficit in the functional compartment. Negative values of iron stores denote iron deficiency and represent the amount of iron that must be returned to the body before iron stores can accumulate.

In this report we estimated iron stores by the method of Cook et al (13), which uses a combination of biochemical measurements of iron status. Different levels of iron stores require different calculations, as noted below and in previous publications (4, 14):

Level 1. This was defined as iron-deficiency anemia (abnormal Hb, < 125 g/L for women and < 140 g/L for men), together with a transferrin saturation < 16% and a serum ferritin concentration < 12 $\mu\text{g/L}$. The following equation was used to calculate iron stores in level 1 patients:

$$\text{Iron stores (in mg)} = -15 \times (\text{mean Hb} - \text{observed Hb})$$

where 15 is the amount of iron (mg) for each 1-g/L reduction in circulating Hb concentration.

Level 2. This was defined as deficiency without anemia. The level 2 algorithm was used for all subjects not fitting the criteria for either level 1 or level 3. Iron stores in level 2 patients were calculated as follows:

$$\text{Iron stores (in mg)} = -80 \times \text{corrective index}$$

where 80 represents one-fifth of the approximated deficit in body iron stores (400 mg) that must occur before Hb concentrations fall below the cutoff point for normal Hb concentrations (see level 1 above); the corrective index was adapted from Cook et

TABLE 1

Experimental data and characteristics of elderly blood donors and control subjects¹

	Donors (n = 9 F, 3 M)	Control subjects (n = 4 F, 4 M)
Age (y)	73 ± 4.5	72 ± 3.1
Weight (kg)	72 ± 8.5	70 ± 10.9
Height (m)	1.66 ± 0.09	1.67 ± 0.134
Iron stores		
Baseline (mg/kg)	8.27 ± 4.25	11.91 ± 4.10
Time of study (mg/kg)	-0.55 ± 3.46	10.69 ± 4.88
Change (mg/kg)	-8.82 ± 4.79	-1.22 ± 2.49
Iron absorption (mg)	4.88 ± 2.35	1.69 ± 0.95
Iron absorption (%)	7.4 ± 3.6	2.5 ± 1.4

¹ $\bar{x} \pm SD$.

al (13). Index values ranging from 0 to 5 are defined in Appendix A. These assigned index values were derived by Cook et al (13), who included erythrocyte protoporphyrin concentrations as well as serum ferritin concentrations and transferrin saturation when deriving these indexes.

Level 3. This was defined as patients with positive iron stores (serum ferritin concentrations > 12 µg/L). In these individuals the following equation was used:

$$\text{Iron stores(mg)} = 400 \times (\ln \text{PF} - \ln 12)$$

where 400 is the proportionality constant, ln is the natural logarithm, and PF is serum ferritin in µg/L.

Statistical analysis

All computations and statistical analyses were done by using SAS version 5.18 (SAS Institute Inc, Cory, NC) on an IBM personal computer. Simple linear regression, Student's t test, and Pearson correlation techniques were used.

Results

Table 1 summarizes the results obtained from 12 donor and 8 control subjects. Figure 1 represents a graphic depiction of the subjects' iron stores determined at entry into the blood donation study, ≈3 y before the present study, and again at the time of the present study. The average number of blood donations by the donor group over this period of time was 13.6 ± 2.3 units. As expected, iron stores were markedly decreased in the donor group, often resulting in negative iron stores. There were minimal changes from baseline iron stores in six of eight control subjects: changes in the other two control subjects (#16 and #17, Fig 1) are unexplained.

The oral iron-absorption test was completed in all subjects. Figure 2 shows the percent iron absorbed in donors and control subjects. The mean (±SD) iron absorbed by the donor group was 7.4 ± 3.6% and 2.5 ± 1.4% for the control group. The difference in iron absorption between these two groups was statistically significant at P = 0.0009. In Figure 3, a statistically significant linear correlation is demonstrated in relating changes from baseline in iron stores (mg/kg) for each subject and percent of iron absorbed, r² = 0.702, P = 0.0001.

BAO ranged from 1.62 to 10.20 mmol/h in the donor group and from 2.01 to 6.71 mmol/h in the control group, all within

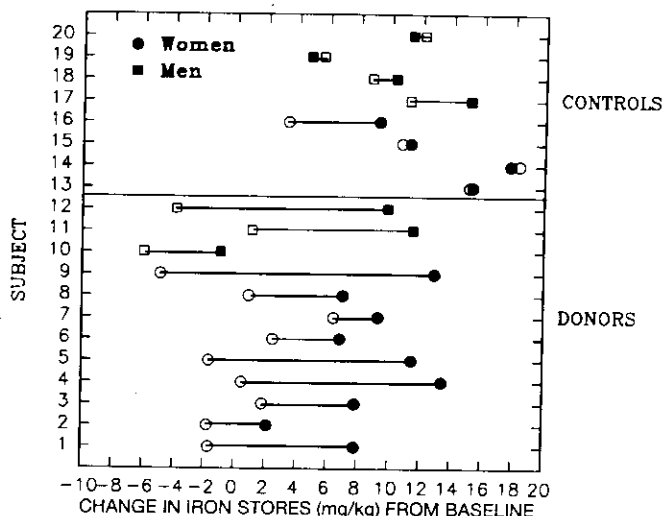


FIG 1. Iron stores determined at baseline (solid symbols) and at the time of the present study (open symbols) for 12 elderly blood donors and 8 elderly control subjects.

normal limits. BAO was normal in 17 of 21 subjects and none were hyposecretors. Because of a failed water-recovery test in two subjects and difficulty in nasogastric placement in two other subjects, BAO was not measurable in two control subjects and two donors. When the possible relationship between gastric acidity and iron stores was evaluated, Figure 4 failed to show any correlation between BAO and baseline iron stores (r = 0.093, P = 0.73) in these normochlorhydric subjects. Iron absorption in one donor was excluded because the subject was an active donor before entry into the blood-donation study and true baseline iron stores were not available.

Discussion

Numerous studies (1, 3, 15) clearly demonstrate an adaptive response in intestinal absorption associated with altered iron

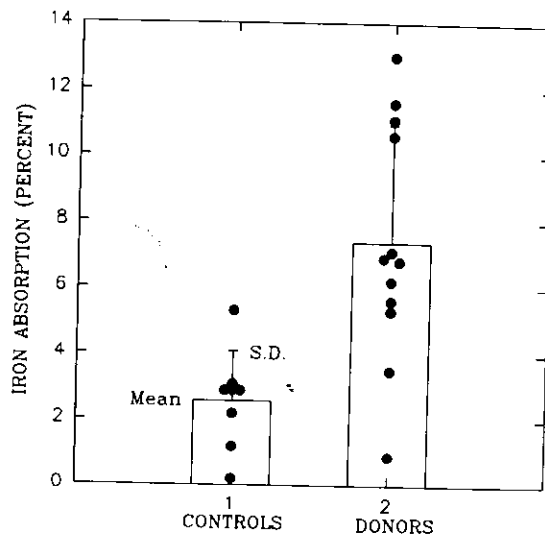


FIG 2. Individual iron absorption values determined in 12 elderly blood donors and 8 elderly control subjects.

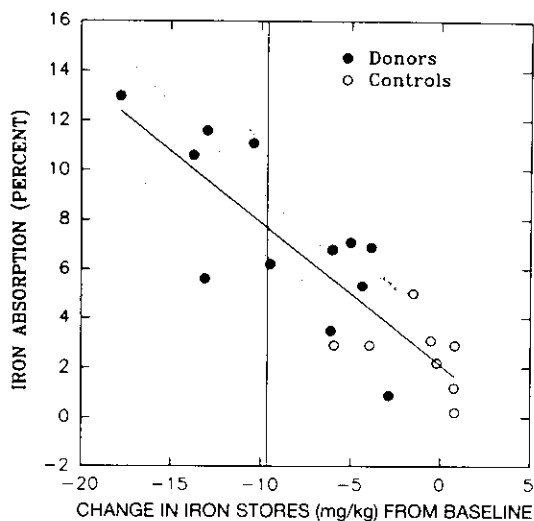


FIG 3. Changes in iron absorption vs change in iron stores from baseline values, with regression line and 95% confidence limits for the regression line. The vertical line represents the point when mean iron stores would have been exhausted for the study population.

stores. As previously noted, this altered absorption was demonstrated in a group of elderly blood donors in whom iron absorption increased as iron stores decreased (4). In nondonors, minimal fluctuations in iron stores occurred over a 2-y period despite relatively large between-subject differences in baseline iron stores. When these differences and their effects on absorption are considered, it seems possible that a setpoint for iron stores exists in each subject, which influences absorption so as to maintain their iron stores at an individually determined amount.

Alternatively, variation in baseline iron stores could reflect the influences of other factors that affect iron metabolism. In exploring this issue, we used nonisotopic methods to assess iron absorption in control subjects and in blood donors known to have depleted iron stores. As noted in the introduction, the role of gastric acid in iron absorption is to promote reduction of ferric iron and solubilization of nonheme dietary iron. Previous work has demonstrated a relationship between achlorhydria and anemia, secondary to impairment of absorption of nonheme dietary iron (16). Therefore, in considering the variation observed in baseline iron stores in healthy elderly subjects (without evidence of iron deficiency), this study also examined BAO and its relationship to iron stores in the same subjects.

When the results of oral iron-absorption tests are analyzed, certain limitations are recognized. Ekenved et al (10) found a good correlation ($r = 0.90$) between increases in serum iron and total amount of iron absorbed after an oral dose of an iron solution. With incomplete transferrin saturation at 3 h after oral iron (observed in all subjects) the elevation in serum iron indicates intestinal absorption, reflecting minimal distribution of iron to the liver during the 3-h test period (17). In addition, the oral iron-absorption test may underestimate the amount of iron absorbed as a result of iron being trapped within enterocytes and the possibility that absorbed iron is immediately taken up by transferrin receptors on target organs. Ekenved et al (10) questioned whether calculations based on the serum iron method could be used to give valid information about the amount of iron absorbed by a single individual, given the large degree of be-

tween-subject variability in results when the test was applied to a mixed population. This variability was also observed in the present study, in the group as a whole. However, when iron absorption was compared in the donor and control groups, a significant difference was observed between the donors (7.4%) and the control group (2.5%) ($P = 0.0009$, Fig 2). Unlike the studies conducted by Ekenved et al (10), in which prior histories of iron stores were unavailable, the present study demonstrated the influence of depletion of stores on iron absorption. These differences in iron stores could explain some of the between-subject variation in absorption despite identical oral doses of iron. When within-subject variation in iron absorption was examined, a mean (\pm SD) value of $2.3 \pm 1.1\%$ was found in one individual examined on five separate occasions. The subject was a healthy 60-y-old male (nondonor) with iron stores of 16 mg/kg. This within-subject variation is similar to the between-subject variation in iron absorption noted in the control population ($2.5 \pm 1.4\%$) and supports the usefulness of the nonisotopic iron-absorption test to assess differences in iron absorption.

Reductions in iron stores were determined by calculating the difference between baseline iron stores at entry to the blood-donation study and at the time of the present study. When iron absorption was compared with the observed change in iron stores, a significant correlation was noted: 70% of the variance in iron absorption could be accounted for by changes in iron stores from baseline values (Fig 3). Simply put, the greater the loss in iron stores, the greater the increase in iron absorption. These findings are consistent with iron absorption adapted to the degree of reduction in iron stores below the setpoint. This does not preclude that other factors may also affect iron absorption.

In addition to the above discussion supporting the use of the serum iron method to assess iron absorption, note that the mean of baseline iron stores in these 20 elderly volunteers was calculated to be 9.72 ± 4.47 mg/kg. Figure 3 shows that when iron stores are adequate and at an assumed steady state, mean absorption is $\approx 2.0 \pm 1.0\%$, increasing in a linear fashion to $\approx 8.0 \pm 1.0\%$ when iron stores approach exhaustion (0 iron stores as represented by the vertical line in Fig 3). These estimates of iron absorption are consistent with previous estimates calculated by

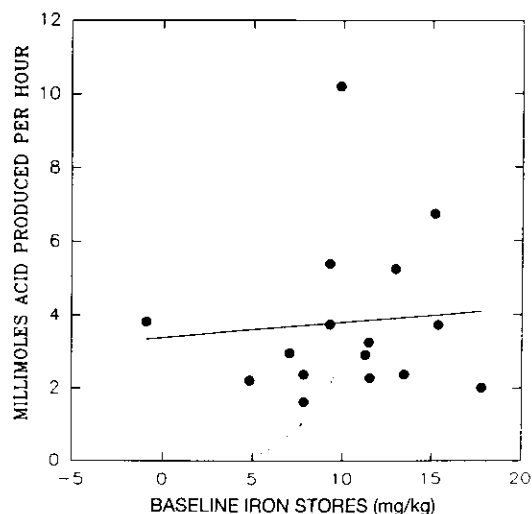


FIG 4. Gastric acid secretion for 16 elderly men and women vs baseline iron stores, with regression line.

assessing donor stores at each donation, while recording their iron intake from diet and supplements (4).

These findings are also consistent with what is previously known about iron metabolism (3). First, regulation of iron absorption is by the gastrointestinal mucosa. Second, body iron stores are the most significant factor affecting iron absorption (1). Biologic adaptation is demonstrated by the relationship between iron stores and iron absorption. The existence of a predetermined setpoint for iron stores and iron absorption noted in these subjects. Elderly subjects, in contrast to younger adults, are more likely to have achieved steady states despite wide between-subject differences in baseline iron stores (13), an advantage in the present study.

Considering the range of variation among the group, the influence on absorption of factors other than a storage setpoint, such as variation in gastric acidity, might explain individual differences in baseline iron stores (4). Gastric acid promotes the solubilization of ferric iron, enhancing nonheme dietary iron absorption, but has no effect on absorption of ferrous or heme iron (18). Previous observations suggest that dietary non-heme iron absorption is significantly inhibited if hypochlorhydria is severe and sustained (5). Therefore, over time, iron status should reflect the effect of hypochlorhydria on dietary iron absorption. In all subjects tested, basal gastric acid secretion was within normal limits. No relationship was seen in these normochlorhydric subjects between variation in BAO and variation in iron stores (ie, a higher BAO was not associated with higher baseline iron stores or vice versa) (Fig 4). Therefore, the variation in iron stores in these normochlorhydric subjects could not be attributed to deficient gastric acid secretion. Thus, iron stores probably were not influenced significantly by iron bioavailability or gastric acidity. On the basis of this hypothesis, one might predict, as seen here, that baseline iron stores would change minimally in nondonors over an extended period of time despite marked initial differences between subjects' iron stores.

In summary, in normochlorhydric subjects, iron absorption appears to be largely determined by an individual's iron storage setpoint. Absorption is regulated so as to maintain iron stores at this predetermined amount. A critical remaining question is to what extent the setpoint is determined by genetic as distinct from environmental influences, and whether or not an individual's setpoint is affected by disease.

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APPENDIX A

Serum ferritin- and transferrin saturation-dependent indexes for calculating iron stores for iron deficiency without anemia¹

Index	Serum ferritin μg/L	Transferrin saturation %
0	≥ 9 but < 12	≥ 16
1.66	≥ 9 but < 12	< 16
1.66	< 9	≥ 16
3.33	< 9	≥ 10 but < 16
5.00	< 9	< 10

¹ Adapted from Cook et al (13).