Iron metabolism in heterozygotes for hemoglobin E (HbE), α-thalassemia 1, or β-thalassemia and in compound heterozygotes for HbE/β-thalassemia 1–3

Michael B Zimmermann, Suthat Fucharoen, Pattanee Winichagoon, Pornpan Sirankapracha, Christophe Zeder, Sueppong Gowachirapant, Kunchit Judprasong, Toshikko Tanno, Jeffery L Miller, and Richard F Hurrell

ABSTRACT

Background: Despite large populations carrying traits for thalassemia in countries implementing universal iron fortification, there are few data on the absorption and utilization of iron in these persons.

Objective: We aimed to determine whether iron absorption or utilization (or both) in women heterozygous for β-thalassemia, α-thalassemia 1, or hemoglobin E (HbE) differed from that in control subjects and compound HbE/β-thalassemia heterozygotes.

Design: In Thai women (n = 103), red blood cell indexes, iron status, non-transferrin-bound iron, and growth differentiation factor 15 were measured, and body iron was calculated. Fractional iron absorption was measured from meals fortified with isotopically labeled (57Fe) Fe sulfate, and iron utilization was measured by the infusion of (58Fe) Fe citrate.

Results: Iron utilization was ≈15% lower in α-thalassemia 1 or β-thalassemia heterozygotes than in controls. When corrected for differences in serum ferritin, absorption was significantly higher in the α- and β-thalassemia groups, but not the HbE heterozygotes, than in controls. HbE/β-thalassemia compound heterozygotes had lower iron utilization and higher iron absorption and body iron than did controls. Nontransferrin-bound iron and growth differentiation factor 15 were higher in the compound heterozygotes, but not in the other groups, than in the controls.

Conclusions: In α-thalassemia 1 and β-thalassemia heterozygotes with ineffective erythropoiesis, dietary iron absorption is not adequately down-regulated, despite a modest increase in body iron stores. In populations with a high prevalence of these traits, a program of iron fortification could include monitoring for possible iron excess and for iron deficiency.


INTRODUCTION

Globally, iron excess occurs mainly in persons with geographically specific genetic mutations that permit the absorption from the diet of more iron than is physiologically needed. Two main types of hereditary iron overload are well recognized: 1) hereditary hemochromatosis, which is seen in populations derived from northern Europe, and 2) the thalassemias and related hemoglobinopathies of South and Southeast Asia, the Middle East, and the Mediterranean (1). Thalassemia mutations are extremely common (2): up to 25% of Thai people are carriers of α-thalassemia, and, in regions of Thailand, Laos, and Cambodia, up to 60% of people are carriers of hemoglobin E (HbE), a hemoglobinopathy caused by a mutation of the β-globin gene. In southern China, which has a population of >350 million, 5% of people are carriers for α-thalassemia and 4% are carriers for β-thalassemia or HbE (3).

Heterozygotes for α-thalassemia 1, β-thalassemia, and HbE typically are asymptomatic and have mild microcytic, hypochromic anemia. In contrast, in thalassemia homozygotes and compound heterozygotes such as HbE/β-thalassemia compound heterozygotes, ineffective erythropoiesis in an expanded marrow stimulates iron absorption even if iron stores are adequate, and this stimulation increases the risk of iron excess when transfusions are given (4, 5). If heterozygotes have some degree of ineffective erythropoiesis and absorb more dietary iron, they may, to a lesser degree than homozygotes and compound heterozygotes, also be at risk of iron excess. In heterozygotes for hemochromatosis, one study reported that iron absorption from a meal with added iron was 3-fold that in controls (6); another study found no differences in absorption (7).

Despite large populations that are heterozygous for thalassemia in countries implementing iron fortification, there are few data on the absorption and utilization of iron in these persons. Earlier studies in β-thalassemia heterozygotes are difficult to interpret because they used oral solutions of 59Fe given with ascorbic acid that overestimate dietary iron absorption and made comparisons without adjustment for differences in iron status (4, 8–11). In countries where thalassemias are common, public

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2 Supported by SUSTAIN (Washington, DC), The Global Alliance for Improved Nutrition (Geneva, Switzerland), the International Atomic Energy Agency (Vienna, Austria), and the Swiss Federal Institute of Technology (ETH) Zürich (Zürich, Switzerland). SF is a senior research scholar of The Thailand Research Fund.
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SUBJECTS AND METHODS

Subjects

The study was carried out in Thai women recruited from the western suburbs of Bangkok. Inclusion criteria were age 18–50 y; premenopausal status; body weight < 65 kg; self-reported nonpregnancy and no plans for pregnancy; no chronic medical illnesses; and desired Hb type: heterozygotes for β-thalassemia; heterozygotes for α-thalassemia 1; HbE heterozygotes; compound HbE/β-thalassemia heterozygotes; and normal HbA. None of the subjects were taking medicinal iron at entry into the study. Twelve percent of the women, distributed through the 5 groups, were taking oral contraceptives. Sample size calculations indicated that 24 women should be included in the control group and the groups heterozygous for β-thalassemia, α-thalassemia 1, and HbE. This number was based on 80% power to detect a 50% difference in iron absorption compared with the control subjects, an SD of 8.2% for log-transformed absorption data from previous absorption studies with the same meal and iron compound in a similar population of Thai women, and a type I error rate of 5%. We also studied 9 compound heterozygotes for HbE/β-thalassemia, a disorder with a clinical phenotype; these persons exhibit clearly ineffective erythropoiesis and were therefore studied as positive controls (4, 5).

Written informed consent was obtained from all subjects. Ethical approval for the study was given by ethical review committees at Mahidol University (Salaya, Nakon Pathom, Thailand) and the Swiss Federal Institute of Technology (ETH, Zürich, Switzerland).

Iron absorption and utilization study

Iron absorption was estimated by using stable-isotope techniques in which the incorporation of $^{57}$Fe and $^{58}$Fe into erythrocytes is measured 14 d after administration (12, 13). If the subjects were taking vitamin or mineral supplements or any other medications, administration was stopped for 2 wk before the study and until the final venipuncture. On day 1, a baseline venous blood sample was drawn after an overnight fast for determination of isotopic composition and confirmation of iron status. The subjects then received a test meal (rice with vegetable soup; see below) labeled with $^{57}$Fe as ferrous sulfate in fish sauce, which was fed under standardized conditions and close supervision. Each test meal contained 3 mg labeled $^{57}$Fe. One hour later, 2 mL of an aqueous solution containing 100 μg $^{58}$Fe as iron citrate was taken in a syringe containing 10 mL of 0.9% saline solution and, via a 100-mL infusion bag leading into a 0.9% saline drip, slowly infused over 50 min (7, 14). The rate of intravenous infusion of iron was based on the estimated 2 μg/min plasma appearance of iron normally absorbed from the gastrointestinal tract (7). No intake of food and fluids was allowed for 4 h after the test meal intake. Fourteen days later, a second venous blood sample was drawn.

Preparation of isotopically labeled iron

$^{57}$Fe was prepared from isotopically enriched $^{57}$Fe by dilution in 0.1 mol H$_2$SO$_4$/L (12). The solution was kept refrigerated in plastic containers, under argon atmosphere. Iron citrate, enriched with $^{58}$Fe, was prepared for intravenous infusion from elemental $^{58}$Fe according to the method previously described (14). The solution was divided in ampoules containing 100 μg Fe, sterilized, and checked for pyrogens. Enrichment of isotopic labels was 95.5% for $^{57}$Fe and 93.1% for $^{58}$Fe. The isotopic composition of the stable-isotope labels was measured by using negative thermal ionization–mass spectrometry (13). Iron-isotope solutions were divided into aliquots and sterilized at the Zürich University Hospital Pharmacy (Zürich, Switzerland).

Test meal composition

The test meal was composed of steamed white rice (50 g dry weight; Jasmine perfume rice; Dragon Phoenix Brand, Bangkok, Thailand), which was served with a vegetable soup containing white cabbage, Chinese cabbage, Thai mushrooms, and steamed carrots in 120 mL of water and seasoned with fish sauce. All ingredients were purchased in bulk and used for the entire study. The food portions were kept frozen until use, and each portion was microwaved on the day of feeding.

Laboratory analysis

Whole blood was transported on ice to the thalassemia laboratory at Mahidol University. Complete blood count and reticulocyte count was done by using the Advia 120 Hematology System with 3-level Advia 120 TESTpoint hematology controls (Bayer, Singapore). Hemoglobin typing for β-globin abnormality was done by using HPLC (Variant Hemoglobin Testing System; BioRad, Hercules, CA) with calibrators and controls provided by the manufacturer. DNA analysis for α-globin abnormalities was done by using a GeneAmp PCR System (Applied Biosystem, Foster City, CA) and a Gel Doc 2000 Gel Documentation System (BioRad, Hercules, CA). Serum ferritin (SF) and serum transferrin receptor (TfR) measurements were made by using an enzyme-linked immunosorbent assay (ELISA; Ramco Laboratories Inc, Stafford, TX) on microplates (CO-DATM Open Microplate System; BioRad); normal ranges: SF, 12–300 μg/L; TfR, <8.2 mg/L. Serum erythropoietin was measured by using an ELISA (R&D Systems, Minneapolis, MN) with controls provided by the manufacturer; the normal range is 3.3–16.6 IU/L. Serum iron (SI), total iron–binding capacity, and transferrin saturation were measured by using colorimetric spectrophotometry (UV-Vis Spectrophotometer 8453; Hewlett-Packard, Palo Alto, CA); the normal range is 9–29 μmol/L for SI, 45–70 μmol/L for total iron–binding capacity, and 30–50% for transferrin saturation. High-sensitivity C-reactive protein was measured by using latex nephelometry (BNProSpec System; Dade Behring Limited, Tokyo, Japan); the normal range is <3 mg/L. SF data from one subject with a CRP concentration > 10 (a HbE/β-thalassemia compound heterozygote) were excluded from the data analysis. For measurement of plasma NTBI, plasma (450 μL) was incubated with 800 nmol nitroliotriacetate/L, pH 7.0 solution (50 μL), at room temperature for 30 min. Plasma proteins were then removed by centrifugation of the
treated plasma with the use of an ultracentrifugation filtration device (30-kDa cutoff, polysulfone type, NanoSep; Pall Life Sciences, Ann Arbor, MI) at 12 000 rpm (10620 g; Hettich Centrifugation, Bach, Germany) and 15 °C for 45 min. The ultrafiltrate was then analyzed by HPLC (15). Nontransferrin-bound iron (NTBI) concentrations in healthy subjects using this method are <0.05 μmol/L. Serum growth differentiation factor 15 (GDF-15) was measured in blinded samples by using a Duoset ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer’s protocol. Whole blood was mineralized by microwave digestion, and iron was separated by anion-exchange chromatography and a subsequent solvent-solvent extraction step into diethylether. Iron was analyzed by negative thermal ionization–mass spectrometry with a magnetic sector field mass spectrometer (Finnigan MAT 262; Thermo Finnigan, Bremen, Germany) equipped with a multicollector system for simultaneous ion beam detection; isotopic dilution calculations were done as described previously (13).

### Statistical analysis

Data were analyzed by using PRISM (version 3; GraphPad, San Diego, CA) and EXCEL (XP 2002; Microsoft, Redmond, WA) software. Body iron stores (BFe) were calculated from the iron status indicators are shown in Table 1. Age, anthropometric, and hematologic indexes by group are shown in Table 1. There were no significant between-group differences in age, height, or weight, except that the HbE group was significantly heavier than the normal hemoglobin and HbE/β-thalassemia groups (P < 0.05). Compared with controls, the α- and β-thalassemia and HbE heterozygote groups had higher RBC concentrations and lower mean corpuscular volume and mean corpuscular hemoglobin (P < 0.05). The α- and β-thalassemia groups had significantly lower hemoglobin than the control subjects (P < 0.02), and most of these heterozygotes were mildly anemic. The HbE/β-thalassemia group had significantly lower RBC, hemoglobin, and mean corpuscular hemoglobin and significantly greater RBC distribution width than did the other groups (P < 0.01 for all).

The iron status indicators are shown in Table 2. The HbE heterozygotes did not differ from the control subjects in any of the iron indexes. The α-thalassemia 1 heterozygotes had significantly (P < 0.05) higher SF, BFe, and erythropoietin (but not NTBI) than did control subjects. The β-thalassemia heterozygotes had significantly (P < 0.05) higher SF, TfR, and BFe (but not NTBI) than did control subjects. The β-thalassemia heterozygotes had slightly higher NTBI than did control subjects, but this difference was not significant. The HbE/β-thalassemia group had significantly higher serum iron, transferrin saturation, SF, TfR, BFe, erythropoietin, NTBI, and GDF-15 concentrations than did the other groups (P < 0.01 for all).

As shown in Table 3, the incorporation of iron into erythrocytes (utilization) from the intravenous iron dose was significantly (P < 0.05) lower in the α- and β-thalassemia groups (but not the HbE group) than in control subjects. Uncorrected fractional iron absorption from the test meal was slightly but significantly (P < 0.05) lower in the β-thalassemia heterozygotes than in the other 4 groups. When corrected for differences in SF, absorption was significantly (P < 0.05) higher in the α- and β-thalassemia groups (but not the HbE heterozygotes) than in control subjects. The HbE/β-thalassemia group had significantly higher absorption and lower incorporation than did the other

### RESULTS

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### Statistical analysis

Data were analyzed by using PRISM (version 3; GraphPad, San Diego, CA) and EXCEL (XP 2002; Microsoft, Redmond, WA) software. Body iron stores (BFe) were calculated from the ratio of TfR to SF (TfR:SF) (16). The amount of 57Fe and 59Fe label present in the blood was calculated from isotope dilution. Circulating iron was calculated from the blood volume and hemoglobin concentration. The amount of stable isotope administered was used to calculate the fractional iron incorporation into erythrocytes (13). The absorption of the oral iron was calculated by dividing the percentage of erythrocyte incorporation of the oral dose by the fractional erythrocyte incorporation of the intravenous dose (7, 14). Results were presented as geometric means ± SDs. Analysis of variance with unpaired t tests and a Bonferroni correction were used to compare the incorporation and absorption data between the groups, and values for iron incorporation and absorption were logarithmically transformed before statistical analysis. In the overall analysis of variance and in the Bonferroni correction, P < 0.05 and P < 0.005, respectively, were considered significant.

### Table 1

| Age, anthropometric variables, and hematologic indexes in Thai women by hemoglobin (Hb) type |
|---------------------------------|-----------------|----------------|-----------------|-----------------|-----------------|
| Subjects (n)                   | Normal Hb       | HbE trait      | α-Thalassemia 1 | β-Thalassemia 1 | HbE/β-thalassemia |
| Age (y)                        | 25              | 26             | 18              | 27              | 9               |
| Weight (kg)                    | 47.8 ± 7.3a     | 53.5 ± 6.3b    | 50.8 ± 8.8c    | 50.4 ± 8.4ab    | 45.4 ± 10.0g    |
| Height (cm)                    | 154.5 ± 5.4     | 154.1 ± 5.7    | 155.8 ± 4.4    | 153.5 ± 5.7     | 152.6 ± 10.0    |
| Red blood cells (×10⁶ cells/mL)| 4.47 ± 0.33a   | 4.78 ± 0.46b   | 5.31 ± 0.44c   | 3.81 ± 0.34d    | 3.2 ± 0.38g     |
| Hb (g/dL)                      | 13.0 ± 1.0a     | 12.4 ± 1.2b    | 11.6 ± 1.0ab   | 11.1 ± 0.7b     | 6.9 ± 1.4c      |
| Range (g/dL)                   | 9.7–14.1        | 9.4–15.8       | 10.1–13.8      | 9.8–12.6        | 4.5–8.3        |
| Subjects with Hb < 12.0 g/dL (n)| 3               | 7              | 13             | 23              | 9               |
| Mean corpuscular volume (µL)   | 83.8 ± 5.8a     | 74.0 ± 6.1b    | 66.6 ± 3.4c    | 63.2 ± 4.0c     | 61.2 ± 6.7d     |
| Mean corpuscular Hb (pg)       | 29.2 ± 2.4a     | 26.2 ± 2.6b    | 21.7 ± 1.0c    | 21.2 ± 1.6c     | 18.5 ± 1.8f     |
| Red blood cell distribution width (%) | 14.4 ± 1.4a   | 15.2 ± 1.4ab   | 15.4 ± 0.7ab   | 15.8 ± 0.8b     | 25.8 ± 2.7g     |

1 Values in a row without common superscript letters are significantly different: P < 0.001 (ANOVA) and P < 0.05 (unpaired t tests with Bonferroni correction).

2 x ± SD (all such values)
grows (P < 0.02 for both). The relation between GDF-15 and erythrocyte incorporation of 58Fe is shown in Figure 1.

In a stepwise regression of all the hematologic variables in Tables 1 and 2 on erythrocyte incorporation of 58Fe, only hemo globin was a significant positive predictor, and SF and GDF-15 were negative predictors (Table 4). In a stepwise regression of these variables on fractional absorption of 57Fe, significant negative and positive predictors were BFe and the red blood cell distribution width, respectively (Table 4).

**DISCUSSION**

Early ferrokinetic studies in β-thalassemia homozygotes reported that erythrocyte incorporation of iron was as low as 15%, whereas it was 75–90% in control subjects (4,8–11). In subjects with β-thalassemia intermedia, iron absorption from labeled meals varied from 26% to 54%, a range that is sharply higher than values in control subjects (4). These studies and others (17) suggested that greater iron absorption and lower utilization in β-thalassemia homozygotes resulted in large increases in body iron that exceeded iron-binding capacity (18, 19). At the same time, there appeared to be a certain component of functional iron deficiency in subjects with thalassemia, because excess iron stores were not entirely available for erythropoiesis (20–22).

If thalassemia heterozygotes partially express the homozygote phenotype (ineffective erythropoiesis and higher plasma iron turnover) and absorb significantly more dietary iron, they may accumulate iron and be susceptible to damage from excess body iron. In previous studies reporting SF or serum iron concentrations (or both) in persons who were heterozygous for β-thalassemia, most found that SF concentrations were similar to or modestly higher than those in healthy persons (23–29). Heterozygotes for β-thalassemia also have higher erythropoietin and TIR concentrations (30, 31) than do control subjects, which indicates a modest increase in erythropoietic drive. Whereas TIR concentrations are higher than those in control subjects, they are lower than those in subjects with iron-deficiency anemia, which suggests ineffective erythropoiesis (32). Previous studies of iron absorption in thalassemia heterozygotes from the 1960s and 1970s were done by using oral solutions of 59Fe given with ascorbic acid (4, 8–11), a method that significantly overestimates true dietary iron absorption (33, 34). Those studies produced mixed results, and a review (8) concluded that mild erythropoietic hyperplasia in heterozygotes for β-thalassemia does not influence iron absorption, and, if iron absorption increases, that change is due to concurrent iron deficiency. The findings of the present study differ. In women heterozygous for α- or β-thalassemia, erythrocyte iron incorporation was ≈15% lower, whereas serum TIR or erythropoietin concentrations (or both)

### Table 2

Iron indexes and biochemical variables in Thai women by hemoglobin (Hb) type

<table>
<thead>
<tr>
<th></th>
<th>Normal Hb</th>
<th>HbE trait</th>
<th>α-Thalassemia 1 trait</th>
<th>β-Thalassemia trait</th>
<th>HbE/β-thalassemia</th>
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<tr>
<td>Serum iron (mmol/L)</td>
<td>15.6 ± 4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.4 ± 7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.1 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4 ± 4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.3 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total iron-binding</td>
<td>50.4 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.6 ± 7.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.3 ± 6.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.9 ± 5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.3 ± 3.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>capacity (mmol/L)</td>
<td></td>
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<tr>
<td>Transferrin saturation (%)</td>
<td>30.7 ± 7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.1 ± 10.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.4 ± 6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.5 ± 7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.8 ± 16.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15 (1–148)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23 (3–112)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>28 (1–142)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52 (2–226)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>877 (206–4040)&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Serum transferrin receptor (mg/L)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6.2 (4.3–15.9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7 (4.0–13.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5 (5.0–10.2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0 (5.4–13.9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.3 (32.6–56.5)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Body iron status (mg Fe/kg body wt)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.1 (10.4–10.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 (5.5–8.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 (9.8–9.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6 (7.8–11.0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.8 (3.9–18.4)&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Serum erythropoietin (IU/L)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4.8 (0.9–30.6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6 (1.8–22.1)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7.0 (2.9–26.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.2 (1.0–33.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.9 (19.2–154.9)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Nontransferrin-bound iron (µmol/L)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.0 (0.5–3.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0 (0.5–3.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0 (0.5–3.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0 (0.5–3.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.3 (12.6–66.9)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>GDF-15 (pg/mL)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>398 (221–1332)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>321 (157–872)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>454 (201–1328)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>414 (190–596)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.09 (1863–61 779)&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

1 GDF-15, growth differentiation factor 15. Values in a row without common superscript letters are significantly different: P < 0.01 (ANOVA) and P < 0.05 (unpaired t tests with Bonferroni correction).
2 Mean ± SD (all such values).
3 Geometric x; range in parentheses (all such values).
4 Median; range in parentheses (all such values).

### Table 3

Iron absorption and erythrocyte iron utilization in Thai women by hemoglobin (Hb) type

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</thead>
<tbody>
<tr>
<td>Erythrocyte 58Fe incorporation</td>
<td>93.1 (82.7, 103.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.2 (79.5, 100.9)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>78.8 (68.4, 89.2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.1 (70.0, 90.3)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.2 (9.3, 33.1)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uncorrected fractional 57Fe absorption</td>
<td>8.7 (3.2, 23.4)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5 (3.4, 16.7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6 (2.8, 11.3)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.5 (2.0, 9.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.5 (4.6, 23.9)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corrected fractional 57Fe absorption</td>
<td>3.0 (0.3, 5.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 (–4.6, 12.6)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>6.3 (1.9, 10.8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.6 (–4.2, 15.9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

1 All values are geometric x; −SD, +SD in parentheses. Iron absorption in each subject was corrected to a value corresponding to 40 µg serum ferritin/L (30). Values in a row without common superscript letters are significantly different: P < 0.01 (ANOVA) and P < 0.05 (unpaired t tests with Bonferroni correction).
were significantly higher, which indicated ineffective erythropoiesis and increased erythropoietic drive. At the same time, iron absorption was less well regulated by the increase in iron stores and was approximately twice that expected for the level of iron stores in persons with normal hemoglobin. This higher iron absorption resulted in modest increases in storage iron as reflected by significantly higher concentrations of SF and Tf.

Both heterozygotes and homozygotes for HbE, the most common form of β-thalassemia, are asymptomatic and minimally anemic, and they have microcytic and hypochromic red blood cells. However, when the β⁰ allele interacts with a β-thalassemia mutation in the compound heterozygous state, a variable and often severe anemia is produced (35) that has evidence of ineffective erythropoiesis and shortened red cell survival (5, 36). In the women in the present study who were heterozygous for HbE, erythrocyte incorporation of ⁵⁸Fe and dietary iron absorption were similar to those in control subjects, which suggests that additional dietary iron absorption and utilization do not differ significantly from those in control subjects. Finally, estimates of body iron (16) in persons with ineffective erythropoiesis may not be valid; however, if we had used SF alone, we would have obtained similar results and relations, and SF concentrations were used to correct the iron absorption values in Table 3.

Although the prevention of iron deficiency through fortification of foods is a recommended strategy (41, 42), there may be potential health risks associated with an overabundant iron supply (43). Because of fears over possible iron overload, iron fortification of flour has been discontinued in Sweden and Denmark (44, 45). Moreover, in developing countries with a high prevalence of hemoglobinopathies (eg, Egypt and Cambodia), public health officials are reluctant to adopt iron fortification (P Winichagoon, R Hurrell, personal communication, 2008). In this context, what is the significance of our findings? In HbE heterozygotes, the most common hemoglobinopathy in Thailand, iron absorption and utilization do not differ significantly from those in control subjects, which suggests that additional dietary iron from universal fortification may be beneficial in populations with low dietary iron intakes. In HbE/β-thalassemia compound heterozygotes, iron absorption and body iron are sharply higher and utilization is impaired, which suggests that additional dietary iron would not be beneficial. But these persons typically are followed clinically, and potential iron excess is monitored and treated. In heterozygotes for α- or β-thalassemia, iron utilization is lower and absorption is not appropriately down-regulated, despite modestly higher concentrations of SF and storage iron. In

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**TABLE 4**

Stepwise regressions of the hematologic variables in Tables 1 and 2 on the dependent variables of erythrocyte incorporation of ⁵⁸Fe and fractional absorption of ⁵⁷Fe

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE</th>
<th>Standardized β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte incorporation of ⁵⁸Fe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>4.423</td>
<td>0.749</td>
<td>0.393</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>−10.249</td>
<td>1.663</td>
<td>−0.338</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GDF-15</td>
<td>−14.220</td>
<td>2.789</td>
<td>−0.336</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fractional absorption of ⁵⁷Fe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body iron stores</td>
<td>−0.041</td>
<td>0.007</td>
<td>−0.563</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Red blood cell distribution width</td>
<td>0.041</td>
<td>0.011</td>
<td>0.358</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 GDF-15, growth differentiation factor 15. Subjects were Thai women (n = 103) who had normal hemoglobin A; were heterozygous for β-thalassemia, α-thalassemia 1, or hemoglobin E (HbE); or who were compound heterozygous for HbE/β-thalassemia. Unstandardized and standardized coefficients are shown.
regions with a high prevalence of these traits, iron should be targeted to groups vulnerable to iron deficiency, such as women and children. If universal iron fortification is implemented, it may be useful to monitor iron stores in groups with lower iron turnover, such as adult males and postmenopausal women. We thank the subjects for their participation in the study and the nursing staff at Mahidol University; we also thank Napon Reabroy (Nakon Pathom, Thailand) and Adam Krzystek (Zürich, Switzerland) for laboratory assistance.

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12. Kastenmayer P, Davidson L, Galan P, Cherouvrier F, Hercberg S, RFH: designed the research; all authors: performed the research and participated in the editing of the manuscript; MBZ, SF, and PW: analyzed the data; and MBZ: wrote the first draft of the manuscript. None of the authors had a personal or financial conflict of interest.