Quantification of body iron and iron absorption in the REDS-II Donor Iron Status Evaluation (RISE) study

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BACKGROUND: Repeated blood donation alters the iron balance of blood donors. We quantified these effects by analyzing changes in body iron as well as calculating iron absorbed per day for donors enrolled in a prospective study.

STUDY DESIGN AND METHODS: For 1308 donors who completed a final study visit, we calculated total body iron at the enrollment and final visits and the change in total body iron over the course of the study. Taking into account iron lost from blood donations during the study and obligate losses, we also calculated the average amount of iron absorbed per day.

RESULTS: First-time/reactivated donors at enrollment had iron stores comparable to previous general population estimates. Repeat donors had greater donation intensity and greater mean iron losses than first-time/reactivated donors, yet they had little change in total body iron over the study period, whereas first-time/reactivated donors had an average 35% drop. There was higher estimated iron absorption in the repeat donors (men: 4.49 mg/day [95% confidence interval [CI], 4.41-4.58 mg/day]; women: 3.75 mg/day [95% CI, 3.67-3.84 mg/day]) compared with estimated iron absorption in first-time/reactivated donors (men: 2.89 mg/day [95% CI, 2.75-3.04 mg/day]; women: 2.76 mg/day [95% CI, 2.64-2.87 mg/day]). The threshold for negative estimated iron stores (below "0" mg/kg stores) was correlated with the development of anemia at a plasma ferritin value of 10 ng/mL.

CONCLUSIONS: These analyses provide quantitative data on changes in estimated total body iron for a broad spectrum of blood donors. In contrast to using ferritin alone, this model allows assessment of the iron content of red blood cells and the degree of both iron surplus and depletion over time.

Iron deficiency affects between 25% and 35% of regular blood donors,[1,2] increasing the risk of adverse outcomes, including hemoglobin (Hgb) deferral, anemia, and side effects like fatigue,[3] neurocognitive abnormalities,[4] pica (compulsive ingestion of substances such as ice), and restless legs syndrome.[5] The amount of iron removed with each blood donation, approximately 250 mg, is high in proportion to stores, representing about 30% of the average body iron stores in men and nearly 80% in women.[6] Recent reports highlight the extended amount of time that is necessary to recover the iron lost from donation. In the REDS-III Hemoglobin and Iron Recovery Study (HEIRS), 67% of unsupplemented donors (including both iron-deficient and noniron-deficient donors at baseline) had not recovered to their baseline ferritin values by the end of the measurement period, 24 weeks later.[7] Methodology to better characterize the kinetics of iron absorption and iron recovery after donation, and how this is altered by oral iron supplementation, is

ABBREVIATIONS: FT/RA = first-time/reactivated; IDE = iron-deficient erythropoiesis; log(sTfR/F) = logarithm of the ratio of soluble transferrin receptor to ferritin; RISE = REDS-II Donor Iron Status Evaluation Study; sTfR = soluble transferrin receptor; TBI = total body iron.

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needed to optimize interventions for maintaining iron balance in blood donors.

Quantitative estimates of body iron stores have been used to characterize iron deficiency in epidemiologic studies.[8,9] In 1990, a simplified index was developed based on the relationship between soluble transferrin receptor (sTfR) and ferritin and correlation of the log(sTfR/ferritin) with iron depletion by serial quantitative phlebotomy.[10] This technique measures the amount of iron available for Hgb synthesis, or "mobilizable" iron stores.[11] Volunteers (six men and eight women; age range, 24-46 years) were bled weekly or biweekly until their Hgb value decreased and plateaued by 2 grams below baseline for 3 weeks and their reticulocytes decreased. This was defined as *iron depletion*. The iron removed by phlebotomy and in blood samples was calculated, and iron loss through the gastrointestinal tract was estimated. As the participants were progressively bled, the investigators observed an inverse relationship with ferritin declining to the lower limit of detection, whereas sTfR shed from iron-deprived erythroid cells increased with greater degrees of iron loss. A regression analysis between iron removed and the log(sTfR/ferritin) ratio revealed a log-linear relationship to body iron stores, providing a quantitative estimate of iron stores based on measurement of the two laboratory values. Using this method, iron stores of 9.82 ± 2.82 mg/kg (776 ± 313 mg) were measured in "normal" men, and stores of 4.87 ± 4.14 mg/kg (309 ± 346 mg) were measured in women in the United States.[8]

In this analysis of data from the REDS-II Donor Iron Status Evaluation Study (RISE),[2,12] we describe a novel method for quantifying changes in iron balance over time using estimated total body iron (TBI) (red blood cell [RBC] iron + iron stores) in donors at the enrollment and final visits. To validate our approach, we compared iron stores in first-time/reactivated (FT/RA) donors in our study with iron stores in the reference populations described above. We also calculated the iron absorption rates of donors participating in the RISE study, taking into account donation during the study and obligate losses. The methods described here should prove particularly useful to measure changes in iron balance in relation to iron dose and the duration of iron intake in iron supplementation studies.

MATERIALS AND METHODS

Study population

The RISE study has been described previously in detail.[2,12] Briefly, the study enrolled two whole-blood donor cohorts, each comprised of approximately equal numbers of men and women: an FT/RA cohort of individuals who had either never given blood before (FT) or had not given a donation in the 2 years before enrollment (RA); and a repeat, "frequent" donor cohort of individuals who had given three or more whole-blood donations in the last year (men) and two or more whole-blood donations (women) in the last year; or equivalent double RBC donations. In total, 2425 donors were enrolled; and, of these, 1334 (55%) returned between 15 and 24 months after their enrollment visit for a final study visit. After accounting for missing samples or laboratory results necessary to calculate TBI at both enrollment and final visits, the cohort for testing (N = 1308) consisted of 181 and 143 FT/RA women and men, respectively, and 478 and 506 repeat women and men, respectively. Among these 1308 donors, there were 8898 visits, of which 600 were Hgb deferrals (based on finger-stick sampling). For the 600 Hgb deferral visits, there were 352 in which we were able to obtain a sample for venous Hgb and body iron measures.

Laboratory testing

Ferritin and sTfR measurements were performed on ethylenediaminetetraacetate plasma frozen at −20° C from a sample obtained at each donation (plasma ferritin concentration is approximately 5% lower than serum).[13] Ferritin was measured using the ADVIA Centaur Ferritin Assay (Siemens Healthcare Diagnostics, Inc.). An immunoturbidimetric assay was used to measure sTfR (Tina-quant Soluble Transferrin Receptor Assay; Roche Diagnostics). Tests were performed by ARUP Laboratories. Paired analysis of results was done for all participants who had both an enrollment sample and a final visit sample (n = 1308). Testing was also performed if a sample was collected at the time of a deferral visit (n= 352).

Determination of iron status

In this study, we considered TBI as reflecting a storage compartment and a functional compartment consisting of circulating Hgb (measured) and marrow and tissue iron (not measured). Storage iron was measured using the methodology developed by Skikne and colleagues[10] and Cook and coworkers,[8] as expressed by the following formula:

$$\text{Iron stores (mg/kg)} = [-\log(\text{sTfR/ferritin ratio}) - 2.8229] / 0.1207.$$

To use this formula, we also converted our Roche Tina-quant sTfR assay values to the sTfR assay values used in Cook and colleagues' assay (known as the "Flowers" assay) using a regression equation based on a published comparison of the two assays: Flowers sTfR = (1.5 * [Roche_sTfR + (0.071 * Roche_sTfR)]) + 0.35.[14] As originally proposed, positive values for iron stores reflected the amount of iron that can be removed from the body without inducing a deficit in the functional compartment. Negative values represented tissue iron deficiency.

For the 1308 RISE donors who completed a final visit and had adequate samples, we calculated TBI and iron stores by cohort using cross-sectional analysis at the enrollment and final visits and the change in TBI from the enrollment visit to the final visit (delta iron). We then determined the amount of iron lost during the course of the study from blood donation and insensible loss and solved for the amount of iron absorbed by the donor, expressed as milligrams of iron per day, using delta iron, iron loss, and the number of days on study.

Key variables

The calculated variables are described below.

1. Calculate TBI at the enrollment and final visits:

$$TBI = body\ iron\ stores + iron\ in\ the\ circulating\ RBC\ Hgb,$$

where *body iron stores* are the iron stores (in mg/kg) for visits in which sTfR is measured * (weight * 0.45359237); *weight* is the weight of the participant (in pounds) based on the most frequent weight reported converted to kilograms; and *iron in the circulating RBC Hgb* is the total body Hgb in grams * 3.4 mg of iron per gram of Hgb.[15]

$$\text{Total body Hgb} = \text{EBV (liters)} \times 10\,\text{dL/L} \times 0.91 \times \text{Hgb (g/dL)},$$

where *EBV* is the estimated blood volume, and *0.91* is a correction factor to convert peripheral to central hematocrit.[16] EBV was calculated using the Nadler formula.[17]Note that iron found in marrow RBC precursors and in tissues other than storage iron, such as cytochromes, transferrin, and myoglobin, was not measured. This functional compartment was considered to be relatively small (approximately 10-12% of total)[18] and stable over the course of the study and was ignored in the calculations of TBI and delta iron (see below).

2. Calculate delta iron:

$$Delta\ iron = (TBI\ at\ final\ assessment) - (TBI\ at\ baseline\ assessment).$$

3. Calculate iron losses during the study:

$$Iron\ loss = total\ loss\ from\ blood\ donations + insensible\ loss + menstrual\ loss (\text{as applicable}),$$

where *total loss from blood donations* is derived by adding up the milligrams of iron lost in each unit of donated RBCs during the period from enrollment visit to the final visit (but does not include loss at the final visit). For whole-blood donation, we calculated the iron lost using the following formula:

$$Iron\ lost\ in\ the\ unit\ (mg) = donated\ whole\text{-}blood\ volume\ (dL) * venous\ Hgb\ (g/dL) * 3.4\ mg\ iron/g\ Hgb.$$

The actual donated whole-blood volume was not collected in the data set. Therefore, we used the volume (in deciliters) of the whole-blood donation using center-specific estimates of donated volumes, including samples. For double RBC units and other RBC apheresis donations, we calculated iron loss based on center-specific reports listing the parameters of their existing apheresis instrumentation and procedures. *Insensible loss* from the gastrointestinal tract was assumed to be 1.0 mg per day * days on study, and *menstrual loss* (for menstruating women) was 0.5 mg per day * days on study. We used menstruation questions from a baseline questionnaire to define women who were and were not menstruating, or we used women aged 50 years or younger to designate probable continued menstrual losses if questionnaire data on menstruation were missing.

4. Calculate iron absorption per day:

$$Iron\ absorbed\ per\ day = delta\ iron + iron\ loss/days\ on\ study.$$

Statistical analysis

All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc.). The arithmetic or geometric means and 95% confidence intervals were calculated for venous Hgb, plasma ferritin, sTfR, log10(sTfR/ferritin), body iron stores (both adjusted and not adjusted for body weight), RBC iron, measured TBI, delta iron, iron loss, and iron absorbed by sex and donor type (first-time and repeat) for both the baseline visit and the final visit. The p value testing for a significant correlation between ferritin and body iron stores was calculated with an analysis of variance using the SAS procedure GLM.

RESULTS

Measurements of iron status at enrollment and final visits

Table 1 shows the distribution of TBI and iron compartments in FT/RA and repeat donors at enrollment. Using this approach, Cook and colleagues estimated body iron stores of 9.82 ± 2.82 mg/kg (776 ± 313 mg) in men and 4.87 ± 4.14 mg/kg (309 ± 346 mg) in young women.[8] Our estimates reveal similar values in RISE FT/RA male donors. Storage iron values were slightly higher in RISE FT/RA women younger than 50 years, which may be due to minor differences in age distribution of the population sampled in the RISE study compared with the more premenopausal-aged population (range, 20-45 years) used in the analysis by Cook and coworkers.[8] As expected, FT/RA women had less TBI and lower iron stores compared

TABLE 1. Iron status indicators at baseline visit by sex and donor status

	Mean (95% CI)		
	Women		
Iron status indicators at baseline visit	<50 years	≥50 years	Men
FT/RA donors*	n = 98	n = 83	n = 143
Venous hemoglobin, g/dL	13.27 (13.08-13.46)	13.63 (13.42-13.84)	14.85 (14.69-15.02)
Plasma ferritin, ng/mL [GM]	31.97 (27.34-37.39)	61.05 (51.94-71.75)	103.84 (92.92-116.05)
Soluble transferrin receptor, mg/L [GM]	2.65 (2.51-2.80)	2.80 (2.64-2.97)	2.74 (2.62-2.86)
Log10[sTfR/ferritin]	1.92 (1.84-2.00)	1.66 (1.59-1.74)	1.42 (1.37-1.47)
Body iron stores, mg/kg†	5.67 (5.02-6.31)	7.81 (7.19-8.44)	9.80 (9.36-10.23)
Body iron stores, mg†	411.1 (354.1-468.2)	590.9 (535.5-646.2)	880.8 (829.1-932.5)
RBC iron, mg	1720 (1657-1782)	1792 (1729-1855)	2588 (2527-2648)
Total measured body iron, mg†	2131 (2028-2234)	2383 (2281-2486)	3469 (3370-3568)
Repeat donors	n = 175	n = 303	n = 506
Venous hemoglobin, g/dL	13.01 (12.88-13.13)	13.37 (13.26-13.49)	14.54 (14.44-14.64)
Plasma ferritin, ng/mL [GM]	15.19 (13.75-16.78)	19.26 (17.94-20.66)	25.04 (23.42-26.77)
Soluble transferrin receptor, mg/L [GM]	3.33 (3.17-3.49)	3.20 (3.10-3.32)	3.28 (3.17-3.38)
Log10[sTfR/ferritin]	2.34 (2.28-2.40)	2.22 (2.18-2.26)	2.12 (2.08-2.15)
Body iron stores, mg/kg†	2.23 (1.77-2.68)	3.21 (2.88-3.54)	4.08 (3.77-4.39)
Body iron stores, mg†	168.9 (134.2203.7)	232.6 (207.4-257.7)	375.6 (345.3-405.8)
RBC iron, mg	1687 (1647-1728)	1712 (1683,1741)	2527 (2494,2559)
Total measured body iron, mg†	1856 (1793-1920)	1944 (1901-1988)	2902 (2848-2956)

* For FT/RA donors, note that total numbers are slightly reduced because of unavailable samples compared with the RISE study. These data reflect only those donors who completed a final visit and had adequate samples for testing (1308/2425 or 54% of donors).

† Body iron stores (mg/kg) = −(log[sTfR/F ratio] − 2.8229)/0.1207 after conversion of sTfR according to Pfeiffer and colleagues;[14] ferritin levels were adjusted up 5%.

GM = geometric mean.

with men. Both men and women who were repeat donors had lower TBI than FT/RA donors, as mainly reflected by lower iron stores. Table 2 lists the values obtained at the final visit, by which time the FT/RA cohort had donated a little over twice per year and the repeat cohort had donoted nearly 3.5 times per year, continuing their previous high frequency. In FT/RA donors, TBI was reduced from enrollment primarily by reduction in stores; whereas, in repeat donors, iron status had already plateaued at a reduced level that was unchanged compared with enrollment levels.

Iron balance and iron absorption in blood donors

The differences in overall iron status among the cohorts according to sex are detailed in Table 3. Greater donation intensity in repeat donors (mean, 4.4 donations in women, 5.2 in men during the study) led to greater mean iron losses than in FT/RA donors (mean, 2.6 donations in women, 2.9 in men), yet repeat donors had little net change in iron status. Maintenance of iron balance (delta close to zero) in the repeat donor group was associated with increased iron absorption. Figure 1 depicts the average iron absorption in men and women from the RISE trial, expressed as milligrams per day while on study. Iron absorption was higher for repeat donors than for FT/RA donors and was approximately equal by sex. Donors were surveyed regarding whether they took iron supplements of any kind during their participation in RISE. Overall, 438 of 1308 donors (34%) responded "yes" to having taken iron supplements. The rate of supplementation was lowest in men (20% in FT/RA donors, 25% repeat donors) and FT/RA women younger than 50 years (27%) and was highest in women who were repeat donors (47%). In a regression analysis, patients who took iron supplements increased their iron absorption by 0.12 mg per day ($p = 0.006$). Iron absorption was increased by 0.43 mg per day for each additional donation per year (data not shown).

We also compared the proportion of donors who had body iron stores ≤0 mg/kg between the enrollment and final visits: iron stores among women who were FT/RA donors increased from 2.2% to 11.1% and, among men who were FT/RA donors, increased from 0.7 to 2.8%; women who were repeat donors were unchanged (17.2%-15.5%; men, 14.0%-14.2%. This represented a fourfold to fivefold proportionate increase in negative iron stores in FT/RA donors and a persisting iron deficit in repeat donors.

Iron status of blood donors deferred for low Hgb

Table 4 depicts the mean venous Hgb values, iron measures, and iron stores in the Hgb-deferred donors during the RISE study. Among the 600 Hgb deferrals, there were 508 in women and 92 in men. This represented an overall Hgb deferral rate (among all visits for those donors who completed a final visit) of 6.7%, which is approximately one-third lower than the overall Hgb deferral rate of 9.5%

TABLE 2. Iron status indicators at final visit by sex and donor status

	Mean (95% CI)		
	Women		
Iron status indicators at final visit	Age <50 years	Age ≥50 years	Men
FT/RA donors*	n = 98	n = 83	n = 143
Venous hemoglobin, g/dL	13.13 (12.89-13.38)*	13.29 (13.01-13.57)	14.62 (14.41-14.83)
Plasma ferritin, ng/mL [GM]	18.92 (16.46-21.76)	28.32 (23.51-34.11)	42.6 (37.29-48.66)
Soluble transferrin receptor, mg/L [GM]	2.79 (2.64-2.94)	2.93 (2.73-3.14)	2.77 (2.64-2.90)
Log10[sTfR/ferritin]	2.17 (2.10-2.24)	2.01 (1.92-2.11)	1.81 (1.75-1.88)
Body iron stores, mg/kg†	3.62 (3.03-4.20)	4.90 (4.10-5.70)	6.56 (6.02-7.10)
Body iron stores, mg†	264.0 (213.5-314.5)	370.9 (309.9-432.0)	584.4 (531.8-637.1)
RBC iron, mg	1703 (1638-1767)	1745 (1682-1808)	2548 (2484-2613)
Total measured body iron, mg†	1967 (1868-2065)	2116 (2014-2219)	3132 (3034-3231)
Repeat donors	n = 175	n = 303	n = 506
Venous hemoglobin, g/dL	13.03 (12.86-13.19)	13.16 (13.02-13.29)	14.37 (14.25-14.50)
Plasma ferritin, ng/mL [GM]	16.53 (14.79-18.48)	20.07 (18.47-21.82)	24.08 (22.425.88)
Soluble transferrin receptor, mg/L [GM]	3.01 (2.87-3.17)	3.01 (2.89-3.13)	3.05 (2.94-3.16)
Log10[sTfR/ferritin]	2.26 (2.20-2.32)	2.18 (2.13-2.22)	2.10 (2.06-2.14)
Body iron stores, mg/kg†	2.86 (2.36-3.37)	3.57 (3.20-3.94)	4.17 (3.83-4.51)
Body iron stores, mg†	212.0 (174.0-249.9)	255.7 (227.8-283.7)	384.9 (352.1-417.7)
RBC iron, mg	1692 (1647-1737)	1684 (1654-1715)	2498 (2463-2534)
Total measured body iron, mg†	1904 (1833-1975)	1940 (1894-1986)	2883 (2824-2942)

* For FT/RA donors, note that total numbers are slightly reduced because of unavailable samples compared with the RISE study. These data reflect only those donors who completed a final visit and had adequate samples for testing (1308/2425 or 54% of donors).

† Body iron stores (mg/kg) = −(log[sTfR/F ratio] − 2.8229)/0.1207 after conversion of sTfR according to Pfeiffer and colleagues;[14] ferritin levels were adjusted up 5%.

GM = geometric mean.

TABLE 3. Change in total body iron, iron loss, and iron absorbed per day (mean and standard deviation), by sex and donor status

	Mean ± SD		
	Women		
Variable	Age <50 years	Age ≥50 years	Men
FT/RA donors	n = 98	n = 83	n = 143
Delta iron, mg	−164 ± 319	−267 ± 327	−336 ± 387
Iron loss, mg	1791 ± 496	1827 ± 503	2003 ± 590
Iron absorbed, mg	1626 ± 430	1560 ± 464	1667 ± 507
Iron absorbed per day, mg	2.80 ± 0.75	2.70 ± 0.77	2.89 ± 0.88
Repeat donors	n = 175	n = 303	n = 506
Delta iron, mg	48 ± 324	−4.31 ± 308	−19 ± 395
Iron loss, mg	2232 ± 491	2140 ± 546	2642 ± 534
Iron absorbed, mg	1170 ± 510	2136 ± 583	2623 ± 588
Iron absorbed per day, mg	3.88 ± 0.89	3.68 ± 0.98	4.49 ± 0.98

*For a worked example of calculations in a blood donor, see Table S1 (available as supporting information in the online version of this paper).

SD = standard deviation; delta iron = total body iron.

in all RISE enrollees and visits. Among the 600 deferrals, there were 352 visits in which we were able to obtain a venous sample to determine the Hgb and iron stores (310 women and 42 men). In addition to the expected decrease in RBC iron because of lower Hgb values, mean iron stores were near zero in these donors, averaging 0.9 mg/kg in women and −0.4 mg/kg in men. Overall, 77.7% of deferred women and 85.7% of deferred men had iron-deficient erythropoiesis (IDE), defined as >2.07 log[sTfR/ferritin].[12,13]

Relationship between iron stores and anemia

Figure 2 illustrates the correlation between iron stores and the prevalence of anemia in RISE donors. It can be seen that anemia (using World Health Organization criteria of venous Hgb <12 g/dL in women and <13 g/dL in men) begins to appear when iron stores are in the negative range and increases sharply to 40 to 58% with progressive deficits in storage iron (Pearson $r^2 = 0.38$; $p < 0.0001$). Because sTfR measurements are not always available, we also wanted to determine whether iron stores could be

estimated in a healthy donor population by ferritin alone. Figure 3 illustrates the relationship between body iron stores (in mg/kg) and ferritin concentration (ng/mL) among women at the baseline visit (n = 659), which we obtained by fitting a regression of body iron stores calculated as described above on ferritin (body iron stores = $\alpha + \beta$[log10(ferritin)]; $r^2 = 0.93$; $p < 0.0001$). A similar linear correlation of iron stores and ferritin level was obtained using data from the male participants (n = 649; data not shown). Iron stores of 0 mg/kg corresponded to a ferritin level of approximately 10 ng/mL. Note that, as ferritin (below approximately 10 ng/mL) approaches the analytical lower limit of the assay (1 ng/mL), the curve is deflected downward, indicating lower iron stores than reflected by the ferritin concentration.

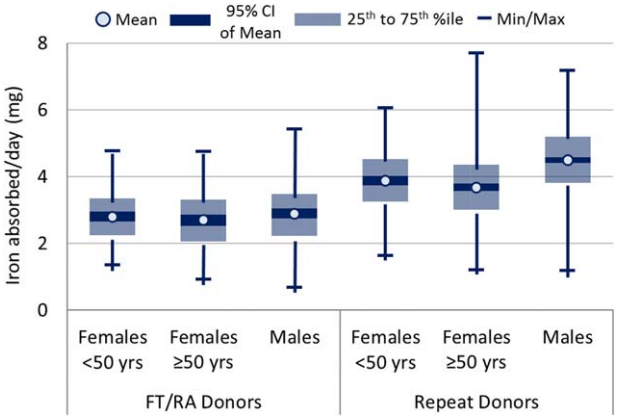

Fig. 1. Distribution of iron absorbed per day during study for women and men, by cohort. For details on the calculation of absorption, see Table S1. [Color figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

We have developed a method to quantify the total iron status of blood donors, taking into account both the largest functional iron reservoir (RBCs) as well as iron stores. Together, these compartments contain from 85 to 90% of TBI.[18] In previous studies, only the storage compartment has been measured,[8,19] which represents only approximately 20% of TBI. Although this approach has been

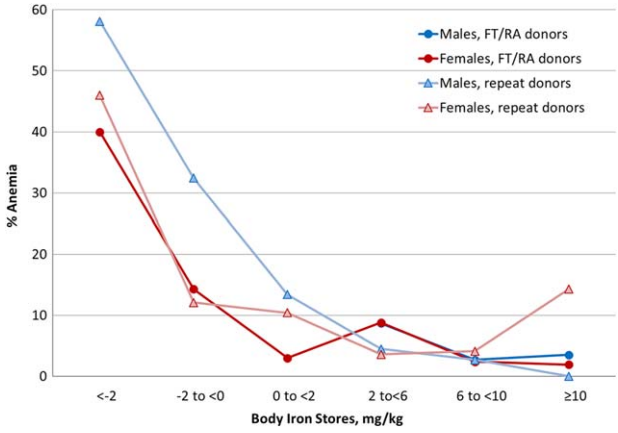

Fig. 2. Relationship between body iron stores and the proportion of donors with anemia as defined by the World Health Organization. Venous hemoglobin values were used to determine the proportion below 13 g/dL (men) and 12 g/dL (women). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4. Iron status measures at deferral visits

	Venous HemoCue Hgb	Ferritin value	sTfR value	Body iron stores, mg*	RBC iron, mg	TBI, mg
Women age <50 years, n = 124						
Mean	11.7	13.9	4.1	32.9	1548.5	1581.4
GM		10.3				
SD	0.7	14.7	1.9	286.6	285.1	472.0
Minimum	10.1	3.0	1.4	−446.1	1149	821.4
Maximum	13.5	122.0	12.2	1098	2784	3789
Women age ≥50 years, n = 186						
Mean	11.8	19.6	4.0	103.3	1524.7	1627.9
GM		12.6				
SD	0.8	21.5	1.5	339.7	254.9	501.1
Minimum	9.8	3.0	1.8	−613.9	1035	767.5
Maximum	14.1	120.0	8.6	1116	2691	3444
Men, n = 42						
Mean	12.1	14	4.6	−41.2	2054	2013
GM		8.9				
SD	1.2	19.2	2.4	364.1	291	550
Minimum	9.1	2	1.5	−738.3	1537	957
Maximum	14.4	118	12.4	762.4	2794	2936

* Body iron stores (mg/kg) = −(log[sTfR/F ratio] − 2.8229)/0.1207 after conversion of sTfR according to Pfeiffer and colleagues[14]; ferritin levels were adjusted up 5%.
GM = geometric mean; SD = standard deviation.

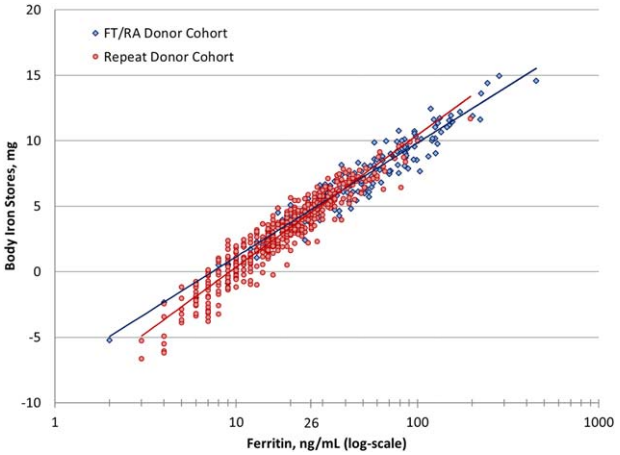

Fig. 3. Relationship between body iron stores and ferritin for women at baseline visit. A similar relationship was observed for males (not shown). A ferritin level of 10 ng/mL corresponds to "zero" iron stores, below which there is a body iron deficit. [Color figure can be viewed at wileyonlinelibrary.com]

employed in populations with stable Hgb levels, it is not a comprehensive assessment of iron balance in a blood donation setting, where acute loss of the Hgb is expected, with or without subsequent recovery. In addition, we found that ferritin values alone may be used to estimate iron stores, as previously reported.[11] However, many blood donors continue to donate in the face of an iron deficit, that is, when they have negative iron stores, which may not be accurately quantified by ferritin levels at the low end of the analytical sensitivity of the assay. Thus, quantifying iron status by the method shown here allows for measurement of the full range of iron balance in blood donors.

We were also able to quantify iron absorption. Accurate estimates of iron balance and absorption should prove useful in studies that employ supplemental iron as a means to mitigate iron deficiency in blood donors, which is being increasingly recognized as an important objective in donor retention and the maintenance of donor health. Revised donor eligibility requirements published in the Code of Federal Regulations increasing the minimum male Hgb level from 12.5 to 13.0 g/dL[20] will result in greater numbers of Hgb deferrals, underscoring the need for blood centers to recognize and prevent anemia in male donors by maintaining positive iron balance.

The body iron stores model has been used in the US National Health and Nutrition Examination Surveys. Mei and colleagues estimated that the prevalence of iron deficiency (iron stores <0 mg/kg) in pregnancy in the United States was 18 ± 1.4% and increased to 30% by the third trimester.[21] The prevalence of anemia commenced in individuals who had estimated iron stores of approximately 0 mg/kg, rising to 32% at body iron stores of −4 mg/kg. In another survey of young women, Cogswell and coworkers observed that the prevalence of iron deficiency was slightly lower than that in the existing model used in the US National Health and Nutrition Examination Surveys (at least two of three abnormal values for free RBC protoporphyrin, ferritin, transferrin saturation), but the iron stores model had better prediction of anemia.[22] In population-based studies involving iron supplementation, the model has demonstrated changes in iron status that were not evident by conventional tests. For example, pregnant Jamaican women who received 100 mg/day elemental iron had significantly higher body iron and iron absorption than women who received 50 mg, although there were no differences in Hgb or sTfR levels.[8]

Previous studies of iron absorption in blood donors were performed before the advent of ferritin measurements or were limited to small and/or nonrepresentative populations. Using dual radiolabelling of both heme and nonheme iron, Hallberg and colleagues studied iron absorption in 31 young, healthy males, including 12 blood donors.[23] In the nondonors (serum ferritin, 91.0 ± 36.9 ng/mL), the average iron absorption was 0.97 ± 0.30 mg/day, whereas blood donors (serum ferritin, 36.8 ± 15.8 ng/mL) absorbed 2.72 ± 1.14 mg/day. Iron from animal sources (heme iron) is absorbed better than nonheme iron (i.e., plant products; approximately 10% absorbed); however, about 90% of dietary iron is from nonheme sources with reduced bioavailability of iron.

Garry and colleagues characterized iron status and absorption in elderly blood donors (mean age, 67.7 years) who donated on average 15 times (approximately every 80 days) over 3.5 years.[24] Initial iron stores, as measured by an older ferritin method described by Cook and coworkers,[9] were 12.45 ± 3.09 mg/kg in men and 12.53 ± 3.24 mg/kg in women and declined progressively, reaching plateau levels after approximately five donations. Mean maximal iron absorption in this intensively donating group was 4.15 mg/day (range, 3.1-4.7 mg/day) in men and 3.55 mg/day (range, 2.7-5.4 mg/day) in women. Deferred donors were analyzed separately (i.e., they were excluded from the primary analysis) and had lower initial iron stores (8.26 ± 2.3 mg/kg in men and 5.61 ± 3.5 mg/kg in women). Mean estimated iron stores were −4.27 mg/kg in deferred men and −4.49 mg/kg in deferred women. In this and another study,[25] ferritin and iron stores were shown to be stable in an individual but with a high degree of intersubject variability; for example, iron stores ranged between 6 and 16 mg/kg in control women who were followed without blood donation over a 2-year period. Factors that determine ability to donate include starting level of iron stores, frequency of donation losses, and iron intake through diet and supplementary sources. A strength of our model is that it allows for the quantification of iron absorption. Although we did not measure interval absorption rates, which were shown to progressively increase and plateau at the maximal rates cited in

the study described above, we recorded similar absorption rates overall in our repeat donors, who averaged 4.49 mg/day in men and 3.75 mg/day in women at the end of the study. A wide range of iron absorption rates were observed, reaching over 7 mg/day in some donors (Fig. 1).

We recognize the limitations of this analysis. Our model did not measure minor tissue iron compartments or marrow, so it was not possible to verify the assumption that they remain static or that they change in proportion to the other compartments. Although we converted the Roche sTfR used here to correspond to the original Flowers sTfR assay value,[14] we did not do the same for the plasma ferritin assay. Ferritin is now calibrated using a reference standard, but such a standard was not used at the time of publication of the original quantitative phlebotomy study by Cook and coworkers, and we are aware of no reported parallel study. Also, the study by Skikne and colleagues was based on iron status changes in only 14 individuals, thus limiting the precision for estimating storage iron.[10] In addition, Cogswell and coworkers questioned the reliability of testing sTfR and ferritin measurements within several weeks of phlebotomy in their study[22]and indicated that there was insufficient time for ferritin to reach steady-state levels that could affect the iron store estimates (in HEIRS, it took approximately 30 days for ferritin to reach its nadir value after phlebotomy).[7] Finally, although we did not measure inflammatory markers, for example, C-reactive protein, that could alter ferritin levels, inflammatory diseases are uncommon in healthy blood donors and thus are unlikely to alter the results.

In our study, donors who completed a final visit and were deferred because of low Hgb concentrations had low average iron stores of 0.9 ± 4.0 mg/kg (75.1 ± 320.9 mg) in women and -0.4 ± 4.4 mg/kg (-41.2 ± 364.1 mg) in men; over three-quarters had IDE, and 51% had negative iron stores. The deferral rate in these donors, 6.7%, was lower than the 9.5% rate in the RISE study overall. The lower rate in donors who completed a final visit is likely due to a culling effect, in which donors who were more susceptible to deferral were unable to reach a final visit. It is possible that differences in iron intake and/or absorption may be at play in donors who do not complete the final visit, and donors who do not maintain their iron balance may be less likely to continue donating. This means our study probably underestimated the iron deficit in deferred donors as a whole. For example, using a different iron stores model with slightly different deferral criteria (Hct 41% in men and 38% in women), Garry and coworkers reported lower average iron stores in deferred elderly donors, as discussed above.[24] It is apparent, however, that donors who are deferred for low Hgb levels operate at a substantial deficit in storage iron.

Our findings also raise the issue of whether the interpretation of negative iron stores (<0 ng/mL), as previously conceptualized by Cook and colleagues, should be equated with functional (or tissue) iron deficiency.[8] In our study, iron stores of "0" mg/kg corresponded in analysis to a ferritin value of approximately 10 ng/mL, which was correlated with the onset and severity of anemia, a finding also noted in the population-based study reported by Cogswell and coworkers.[22] However, our previous work[12,13] and other studies in blood donors[26] have indicated that higher ferritin values (22-26 ng/mL) serve as a more sensitive threshold for IDE and delayed Hgb recovery that better signifies functional iron deficiency. This difference appears to be the result of an inherent bias in the body iron stores model that overestimates iron stores, because by definition to be included in the study that led to this model, subjects had to develop and maintain anemia, which was defined by Cook and colleagues as iron stores of "zero." As shown by the studies mentioned above, tissue iron deficiency impairs the ability to make RBCs and this occurs initially at higher ferritin values, before the onset of frank anemia. Only when Hgb synthesis is sufficiently reduced for the individual to become anemic, that is, from approximately 15 g/dL, falling to 13 g/dL among men and approximately 14 mg/dL, falling to 12 g/dL among women, does an individual meet the criteria set by the body iron stores model, which equates to "zero" iron stores.[8,10] Accordingly, a ferritin level of 26 or better reflects functionally deficient or "absent" iron stores, and a ferritin of 10 ng/mL correlates to the development of anemia.

Our study describes quantitative data on changes in estimated TBI (RBC iron + iron stores), and iron absorption in a broad spectrum of blood donors. We have shown that frequent blood donors start with comparable levels of storage iron as reported in the general population, lose iron from donating while increasing iron absorption, either reaching a new steady state and/or being deferred. Both low iron stores and the relatively low absorption rate described in this and other studies accounts for the high prevalence of iron deficiency in frequent blood donors. While ferritin remains the single best operational measure for assessing iron status blood donor populations, the TBI model offers the enhanced capability to assess the degree of both iron surplus and depletion, which can be analyzed as a continuous variable in determining iron balance and is well-suited to assess the impact of mitigation measures such as iron supplementation in blood donor studies.[27]

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Iron balance worksheet.