Published in final edited form as:

Transfusion. 2012 April ; 52(4): 702–711. doi:10.1111/j.1537-2995.2011.03401.x.

Iron Deficiency in Blood Donors: The REDS-II Donor Iron Status Evaluation (RISE) Study

Ritchard G. Cable¹, Simone A. Glynn², Joseph E. Kiss³, Alan E. Mast^{4,5}, Whitney R. Steele⁶, Edward L. Murphy⁷, David J. Wright⁶, Ronald A. Sacher⁸, Jerry L. Gottschall⁴, Leslie H. Tobler⁹, Toby L. Simon¹⁰, and for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II)

¹New England Region, American Red Cross Blood Services, Farmington, CT

²Transfusion Medicine and Cellular Therapies, National Heart, Lung and Blood Institute, Bethesda, MD

³Institute for Transfusion Medicine, Pittsburgh, PA

⁴Blood Center of Wisconsin, Milwaukee, WI

⁵Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI

⁶Westat, Rockville, MD

⁷University of California, San Francisco and Blood Systems Research Institute, San Francisco, CA

⁸Hoxworth Blood Center, University of Cincinnati Academic Health Center, Cincinnati, OH

⁹Blood Systems Research Institute, San Francisco CA

¹⁰CSL Plasma, Boca Raton, FL

Abstract

Background—Blood donors are at risk of iron deficiency. We evaluated the effects of blood donation intensity on iron and hemoglobin in a prospective study.

Methods—Four cohorts of frequent and first time or reactivated blood donors (no donation in 2 years), female and male, totaling 2425 were characterized and followed as they donated blood frequently. At enrollment and the final visit, ferritin, soluble transferrin receptor (sTfR), and hemoglobin were determined. Models to predict iron deficiency and hemoglobin deferral were developed. Iron depletion was defined at two levels: Iron Deficient Erythropoiesis (IDE) [$\log(\text{soluble transferrin receptor/ferritin} - 2.07)$] and Absent Iron Stores (AIS) (ferritin < 12 ng/mL).

Results—Among returning female first time/reactivated donors, 20% and 51% had AIS and IDE at their final visit, respectively; corresponding proportions for males were 8% and 20%. Among female frequent donors who returned, 27% and 62% had AIS and IDE, respectively, while corresponding proportions for males were 18% and 47%. Predictors of IDE and/or AIS included a higher frequency of blood donation in the last 2 years, a shorter interdonation interval, and being female and young; conversely, taking iron supplements reduced the risk of iron depletion.

Corresponding Author: Ritchard Cable, M.D., New England Region, American Red Cross Blood Services, 209 Farmington Avenue, Farmington, CT 06032, Phone: 860-678-2860, Fax: 860-676-8093, CableR@usa.redcross.org.

The remaining authors declare that they have no conflicts of interest relevant to the manuscript.

Predictors of hemoglobin deferral included female gender, Black race and a shorter interdonation interval.

Conclusions—There is a high prevalence of iron depletion in frequent blood donors. Increasing the interdonation interval would reduce the prevalence of iron depletion and hemoglobin deferral. Alternatively, replacement with iron supplements may allow frequent donation without the adverse outcome of iron depletion.

Keywords

Donors; Hematology – Red Cells; Blood Center Operations

Introduction

Nine and a half million volunteer blood donors give the “gift of life” every year in the U.S. and should do so in a setting that limits potentially adverse health consequences. Whole blood donors can donate every eight weeks and frequent donors are at risk of becoming iron depleted.^{1,2} Studies by Finch³ and Simon¹ in the 1970’s and 1980’s showed that serum ferritin decreased in association with blood donation. Since blood donor iron depletion has been tentatively linked to a variety of non-hematologic symptoms, such as cognitive changes, pica and restless leg syndrome,⁴ prevention of significant iron depletion in blood donors is desirable. However, the potential beneficial effect of blood donation on cardiovascular disease⁵ and cancer mortality⁶ needs to be balanced against these possible negative effects in considering any significant changes in blood donor management.

The magnitude and the predictors of iron depletion in blood donors are not well characterized, especially considering the changes in eligibility criteria and increased blood volume drawn that have been implemented in the last several decades. Since the studies by Finch and Simon were conducted, most blood centers have reduced the required hemoglobin level for males from 13.5 g/dL to 12.5 g/dL and increased the container blood volume from 450 to 500 mL. Also polymorphisms in transferrin⁷ and the HFE genes^{8,9} have been described. These genetic markers might define “at risk” and “protected” donor groups with respect to iron depletion. None of the previous studies of iron depletion in blood donors assessed the influence of race/ethnicity or dietary intake. Finally, the availability of new tests of body iron status provides opportunities to detect or prevent this problem.

For these reasons, the National Heart, Lung, and Blood Institute’s (NHLBI) Retrovirus Epidemiology Donor Study – II (REDS-II) program conducted the REDS-II Donor Iron Status Evaluation (RISE) study whereby blood donors were followed prospectively for 15–24 months to provide an in-depth and contemporary evaluation of iron status in a US blood donor population.

Methods

The RISE study was conducted between 2007 and 2009 at the six REDS-II blood centers which represent geographically and demographically diverse donor populations and account for over 8% of annual US blood collections.

Study Population

The study population consisted of 2425 whole blood or double red cell donors 18 years or older. Four cohorts were established and followed for a maximum of 24 months (Table 1): Two first time/reactivated (FT/RA) donor cohorts consisting of men and women who had either never given blood before (FT) or had not given a donation in the 2 years prior to

enrollment (RA), and two frequent donor cohorts consisting of men who had given the equivalent of 3 and women who had given 2 red cell units in the last year. There were no meaningful differences in iron measurements between first time and reactivated donors at enrollment when controlling for other factors.¹⁰

Only individuals who successfully donated whole blood or double red cell units at their enrollment visit were recruited. Enrollees agreed to return to donate red cell components frequently in the next 24 months, provide blood samples at each visit, and complete a baseline and final questionnaire. The RISE study protocol and donor consent forms were approved by each participating institutional review board.

Data Collection and Donor Management

At enrollment, a self-administered questionnaire (Appendix A) was used to collect donor information such as use of iron supplements and smoking history.¹⁰ Other donor information including blood donation history was compiled from donor and deferral databases, abstracted from blood center records. “Total red cell units donated” was calculated as the number of whole blood donations plus twice the number of double red cell donations plus the number of other red cell donations by apheresis.

Enrolled donors were asked to donate frequently and were routinely recruited. A donor was deferred by current standards^{11,12} if their hemoglobin was < 12.5 g/dL or hematocrit < 38% using routine fingerstick methods (which differed among the six centers).

Beginning at least 15 months following enrollment, donors were recruited for a final visit where a final questionnaire inquiring about changes since the enrollment questionnaire was administered.

Laboratory Testing

Blood samples were collected at all enrollment and final visits, and efforts were made to collect samples at every interim visit. Venous hemoglobin was determined on all collected samples.¹⁰ All enrollment and final visit samples as well as selected interim visit samples were screened for plasma ferritin and soluble transferrin receptor (sTfR) as previously described.¹⁰ Due to budget constraints, only interim samples from selected groups of donors: FT/RA donors upon return, donors deferred for hemoglobin, and, at centers A, D and E, selected frequent female donors considered to be at increased risk for iron depletion were tested.^{1,3} In addition, at enrollment the C282Y and H63D mutations in the HFE gene,⁹ and the G277S mutation of the transferrin gene (which predisposes to iron deficiency anemia)⁷ were determined as previously described.¹⁰ Post-donation venous hemoglobin values (Post vHb) representing approximately 14% of all samples were converted to estimated pre-donation values (Pre vHb) using the formula $\text{Pre vHb(g/dL)} = \text{Post vHb} + 0.8423 - (0.002035 \times \text{Weight (lbs)})$.¹³

Ascertainment of Iron Status Outcomes

The three main outcomes, Absent Iron Stores (AIS), Iron Deficiency Erythropoiesis (IDE) and hemoglobin deferral, were determined as follows. Body iron stores were measured by testing for plasma ferritin and soluble transferrin receptor (sTfR) at all enrollment and final visits and at selected interim visits. A subject was then classified as having absent iron stores (AIS) at any visit if their plasma ferritin was less than 12 ng/mL. This cutoff is a highly specific indicator of iron deficiency that reflects absent tissue and bone marrow iron stores but lacks sensitivity.^{14, 15, 16, 17} Measurement of the soluble transferrin receptor (sTfR) is a sensitive measure of functional iron deficiency.^{18,19} Use of the ratio (sTfR/ferritin) appears to provide high sensitivity in the detection of iron deficient erythropoiesis.^{14, 16} Iron

deficient erythropoiesis (IDE) was defined as the log of the ratio of soluble transferrin receptor (sTfR) to ferritin [$\log(\text{sTfR}/\text{Ferritin})$] ≥ 2.07 , corresponding to the 97.5 percentile of the distribution of the log (sTfR/ferritin) in FT/RA males at enrollment.

Statistical Analysis

Among 2,425 enrolled donors, 1,334 donors made a final donation visit at least 15 months after enrollment allowing for a comparison of iron status (AIS, IDE) and venous hemoglobin between enrollment and final visits using paired analyses. The primary analysis, however, included data on all donations given by the 2,425 donors at various time points. Thus, multivariable repeated measures logistic regression models with either hemoglobin deferral, AIS or IDE as outcomes at each visit were constructed (SAS 9.2 (2008) SAS Institute Inc, Cary NC). Appendix B summarizes variables included in the models which are potentially important predictors of AIS, IDE or hemoglobin deferral, and also indicates their association with iron status at enrollment.¹⁰ The models also included donation variables that changed at each donation; “time since last donation (in weeks)”, and “number of donations in the past 24 months” (interval reset at each donation). The models included age-by-gender and weight-by-gender interaction terms.

Results

Enrollment and Donation Activity

The cross-sectional enrollment data has previously been reported.¹⁰ RISE enrolled 2425 donors. At enrollment, AIS and IDE were present in 6.4% and 24.7% of FT/RA women, 0% and 2.5% of FT/RA men, 27.1% and 66.1% of frequent women, and 16.4% and 48.7% of frequent males.¹⁰

Of all 2425 enrolled donors, 89% returned one or more times during the next 15–24 months, with 1334 donors completing a final visit (Table 1). Donors made 12,695 total visits of which the remaining 8936 visits were interim visits. 11.6% of red cell donations were of double red cells and 0.7% were other red cell apheresis donations. Frequent donors returned more than FT/RA donors (97% vs. 75%), were enrolled in the study longer and donated more total red cell units during the study period, 4.4 and 5.2 units for frequent female and male donors and 2.6 and 2.9 units for female and male FT/RA donors, respectively. (Table 1) On average, returning frequent female and male donors donated at a rate of 3.1 and 3.6 red cell units annually while returning FT/RA donors donated at a rate of 2.2 and 2.4 red cell units annually, respectively. The increased return rate and donation frequency of frequent donors compared to FT/RA donors was expected. However, the participation of FT/RA donors was excellent, so that the effect of non-returning donors on the overall group results would be expected to be minimal.

Predicting Iron Deficiency and Hemoglobin Deferral

Table 2 shows the prevalence of hemoglobin deferral among visits by returning donors, while Table 3 identifies the distribution of visits with ferritin and sTfR test results among all visits and the observed prevalence of IDE and AIS at these visits by demographics and donation intensity.

Multivariable models were developed to identify independent predictors of developing AIS, IDE, and hemoglobin deferral during the study. Adjusted odds ratios (ORs) are presented in Table 4 and Figures 1 and 2. Independent predictors of hemoglobin deferral were female gender, younger age in women (but not men), Black race, and shorter time since the last donation. There were also significant differences in hemoglobin deferral rates by blood center. Figure 1 shows the relationship between donation interval and odds for hemoglobin

deferral. Donors had generally lower odds of deferral as the time since their last red cell donation increased. ORs of donors attempting to donate at least 15 weeks after their last donation (relative to reference group of donors attempting to donate at least 26 weeks after their last donation) were not significant (except for week 17) whereas ORs for donors attempting to donate 8–14 weeks after their last donation were significant and in the range 2.1–2.7. While this trend was present in females and all donors, there were too few male deferrals to separately evaluate if trends in males differed from females.

Similar models for predicting AIS and IDE at visits were developed. As can be seen in Table 4 and Figure 2, female gender and younger age in women were highly significant predictors of AIS and IDE, as was time since their last red cell donation, similar to the hemoglobin deferral model. Although the ORs for AIS were higher than in the hemoglobin deferral model, they also trended lower at about 14 weeks since their last donation, and even lower at 19–20 weeks where they approached unity (Figure 2). Comparable data were obtained for predicting IDE (data not shown).

Unlike the hemoglobin deferral model, the number of donations in the last 2 years was a highly significant predictor of AIS and IDE. For example, donating more than 4 red cells in 2 years results in ORs of 5–9 for AIS and IDE, compared to first time donors. There was a significant protective effect of self-administered iron supplements (AIS OR=0.6 (95% CI: 0.5–0.7); IDE OR=0.7 (95% CI: 0.6–0.8). There was a reduced risk of iron depletion in previous or current smokers (ORs from 0.5–0.8). Significant differences by center were also seen. Less consistent effects were seen for race/ethnicity and HFE genotype (Table 4), and (not shown) recent (< 1 year) but not remote pregnancy. Menstrual status alone was not independently significant in any of the three models.

Changes in Iron Status and Donor Hemoglobin during RISE

A significantly higher proportion of FT/RA donors had iron depletion at their final visit compared to their enrollment visit (all p-values, $p < 0.001$, Figure 3, Panels A and B). Of the 181 FT/RA females who completed an enrollment and final visit, 5% had AIS and 22% had IDE at the enrollment visit, compared to 20% and 51% at the final visit. For the 143 FT/RA males with both visits, 0% and 3% had AIS and IDE at the enrollment visit, versus 8% and 20% at the final visit. The proportion with venous hemoglobin below 12.5 g/dL also increased significantly from enrollment to final visit ($p < 0.001$) for FT/RA females (from 11% to 25%), and increased, but not statistically significantly ($p = 0.07$), for FT/RA males (from 1% to 5%, Figure 3, Panel C). In contrast, the iron status and venous hemoglobin of frequent donors was relatively unchanged during follow-up (Figure 3, Panels A–C).

Discussion

Our data demonstrate a high prevalence of iron depletion in frequent blood donors and a strong association between prior donation intensity and the time since last donation and iron depletion. We also found that iron depletion develops in a high proportion of returning FT/RA donors. In 2006, 9.55 million allogeneic donors successfully gave blood, 6.80 million of whom were repeat donors, providing the equivalent of 1.7 donations per donor per year.²⁰ Our FT/RA donors at their final study visit roughly mimic such repeat donors. Figure 3 Panel B shows that 50% of FT/RA females and 20% of FT/RA males have IDE. Hence, we project approximately 35% (2.4 million) of U.S. repeat donors have IDE, illustrating the importance of iron depletion in blood donors.

Three previous cross-sectional studies have demonstrated that regular blood donors are iron depleted.^{1,3,16} Recently, Rosvik and colleagues conducted a short longitudinal study modeling variables associated with a drop in donor hemoglobin and ferritin at the second to

fourth donations following 893 first time donations.²¹ They also observed a decrease in ferritin in both genders, modified by increasing interdonation intervals. RISE provides the largest and most comprehensive longitudinal study to date of blood donor iron status and also includes an assessment of the impact of a large number of variables possibly related to iron balance.

Two distinctly different donor groups comprised the RISE study: First time and reactivated donors (iron stores initially unaffected by blood donation) and frequent donors were asked to return more often than typical repeat donors who in aggregate donate 1.7 times a year.²⁰ Based on the enrollment criteria for RISE, approximately 54% of all donors giving allogeneic donations at the six REDS-II blood centers during the enrollment period were eligible for enrollment in RISE (38% of repeat donors and all first time and reactivated donors - reference²² and unpublished data). These four RISE cohorts represent a population of donors at higher risk of iron depletion considering their high frequency of donation. Indeed, we found that a significant number of returning first time/reactivated donors (who on average donated 2–3 times during the study) developed iron depletion within 15–24 months. On the other hand, iron status changed relatively little among our repeat donor cohorts.

Previous donation intensity was the most important predictor of AIS and IDE at enrollment¹⁰ and at any subsequent visit. This attests to the approximate 230 mg iron loss associated with every donated red cell unit. Nearly as important was the interval since the last red cell donation; donors who attempted to return to donate within approximately 14 weeks of their last donation had significantly higher odds of AIS or IDE than donors returning between 14–18 weeks, and, even more so, than donors returning at least 19–20 weeks after their last donation.

Deferral for low hemoglobin is the most common cause of blood donor loss, particularly for females. Nearly 10% of all REDS-II blood donation visits (17.7% females; 1.6% males) result in a deferral for a low hemoglobin or hematocrit.²³ In RISE, the vast majority of these deferrals (77%) were associated with iron deficiency. Time since last red cell donation was a highly significant factor for hemoglobin deferral, although the ORs were somewhat lower than observed for AIS and IDE. ORs became non-significant at 14 weeks, suggesting that about 3–4 months is required for the bone marrow to replenish lost red cells, likely limited by the rate of gastrointestinal absorption of iron. Unlike the AIS and IDE models, the number of previous donations in the last 24 months was not predictive of hemoglobin deferral.

As observed previously, female gender and younger age in women were important predictors of AIS, IDE, and hemoglobin deferral.^{1,3} The protective influence of higher weight on the development of AIS/IDE is not surprising, since a blood donation represents an increasing percentage of red cell mass in smaller donors. The increased odds of deferral for low hemoglobin attributed to Black race are consistent with lower normal hemoglobin in this population.²³

Smoking (past or present) was associated with a lower incidence of iron depletion in RISE blood donors. One cross-sectional study in women of menstrual age found that smoking was associated with lower sTfR but not higher ferritin in a multivariable model.²⁴ Another multivariable model in middle-aged Danes, in contrast, found a negative association between ferritin and smoking.²⁵ The only prior blood donor study was in 138 “super donors”, where smoking was associated in a univariable analysis with reduced ferritin levels.²⁶ Our finding is in the opposite direction and does not appear to have been previously reported. Smoking is relatively uncommon in blood donors (12.8% in RISE versus 17.7% in

the general population²⁷) and may serve as a marker of some other unidentified behavioral factor such as unusual alcohol consumption which may affect iron stores. It is also possible that smoking has a previously unidentified direct biologic effect on iron balance. The lack of an effect of smoking on hemoglobin deferral was also unexpected, since smoking is known to increase hemoglobin levels.²⁸

Blood Center was an independent predictor of AIS, IDE, and hemoglobin deferral in our three multivariable models. We have previously reported the influence of blood center on enrollment AIS and IDE in RISE donors.¹⁰ The multivariable models we used in the enrollment study and in the longitudinal follow-up study here should have controlled for the influence of demographic differences, blood donation intensity, smoking, and iron supplementation differences. We also reviewed possible center operational differences that could explain the iron results, but could identify none. It is possible that unrecognized methodological differences existed or that the statistical model we used did not adequately control for underlying donor or donor behavioral differences between centers. Center differences in hemoglobin deferral rates are frequently observed in blood center practice, presumably due to methodological differences in the fingerstick and hemoglobin/hematocrit procedures used. Since there was no apparent concordance between the ORs for AIS and IDE and those for hemoglobin deferral among centers (see Table 3) it is likely that these technical differences, rather than differences in donor iron status, explain the blood center hemoglobin deferral differences observed.

The reduction in the OR for AIS and IDE for donors taking iron supplements is encouraging. Advice on iron was not provided to RISE participants. Iron supplements were reported to be taken by 39% of RISE donors at enrollment,¹⁰ with the proportion not increasing during the study (data not shown). This finding suggests that a program to provide iron supplementation to frequent blood donors might be possible and if acceptable to donors should improve iron stores, as supported by several small trials.²⁹ However, larger trials are needed to further evaluate the effectiveness of such strategies, as well as their operational feasibility. The impact of limiting whole blood donation frequency to every 3–4 months also needs to be evaluated, since such a change could significantly impact blood supplies. A dual approach combining iron supplementation with prolongation of the minimum interdonation interval may be a viable donor strategy which warrants further evaluation, as does evaluation of each intervention alone.

In summary, our large, prospective study of iron status in a US blood donor population demonstrates that, under current donation practices, iron deficiency is highly prevalent in frequent blood donors, especially women of younger age. Interestingly, we did not find that genetic (HFE), race/ethnicity or dietary factors appeared to have a major impact on the frequency of iron depletion. However, because donors under the age of 18 were not recruited in RISE, the impact of blood donation on iron balance in younger blood donors still needs to be assessed.

Optimizing blood donor safety is of paramount importance. Our findings should stimulate additional studies that evaluate whether iron depleted donors demonstrate clinical manifestations and also assess the overall public health significance of iron depletion. Comprehensive trials of changes in blood center practices are also warranted. These trials should include assessment of their feasibility in routine donor management and in maintenance of an adequate blood supply.

Acknowledgments

The authors thank the staff at all six participating blood centers. Without their help, this study would not have been possible. The Retrovirus Epidemiology Donor Study - II (REDS-II Study Group) is the responsibility of the following:

List of participating REDS-II investigators:

Program Office: George J. Nemo, PhD (National Heart, Lung, and Blood Institute, NIH, Bethesda, Md.)

Study Coordinating Center: Jane Schulman, PhD; Melissa R. King, MSPH (Westat, Rockville, Md.)

Central Laboratory: Michael P. Busch, MD, PhD; Phillip Norris, MD (Blood Systems Research Institute, San Francisco, Ca.)

Study Investigators and Blood Centers participating in this study:

American Red Cross Blood Services, New England Region: Ritchard G. Cable, MD; Jorge A. Rios, MD; Richard J. Benjamin, MD;

American Red Cross Blood Services, Southern Region/Department of Pathology and Laboratory Medicine, Emory University School of Medicine: John D. Roback, MD;

Hoxworth Blood Center, University of Cincinnati Academic Health Center: Ronald A. Sacher; Susan L. Wilkinson, EdD; Patricia M. Carey, MD;

Blood Centers of the Pacific, University of California San Francisco, Blood Systems Research Institute: Edward L. Murphy, MD; Brian S. Custer, PhD; Nora V. Hirschler, MD;

The Institute for Transfusion Medicine/LifeSource Blood Services: Darrell J. Triulzi, MD; Ram M. Kakaiya, MD; Joseph E. Kiss, MD;

Blood Center of Wisconsin: Jerry L. Gottschall, MD; Alan E. Mast, MD, PhD.

This work was supported by NHLBI contracts N01-HB-47168, -47169, -47171, -47172, -47174 and -47175

Alan Mast has received payment for lectures from Siemens Corporation; Ronald Sacher is a consultant to Caremark and Guidepoint Global Corporations, provides expert testimony, and has received payment for lectures from GSK and Talecris Corporations.

References

1. Simon TL, Garry PJ, Hooper EM. Iron stores in blood donors. JAMA. 1981; 245(20):2038–2043. [PubMed: 7230400]
2. Mast AE, Foster TM, Pinder HL, Beczkiewicz CA, Bellissimo DB, Murphy AT, et al. Behavioral, biochemical, and genetic analysis of iron metabolism in high-intensity blood donors. Transfusion. 2008; 48(10):2197. [PubMed: 18657084]
3. Finch CA, Cook JD, Labbe RF, Culala M. Effect of blood donation on iron stores as evaluated by serum ferritin. Blood. 1977; 50(3):441–447. [PubMed: 884321]
4. Newman B. Iron depletion by whole-blood donation harms menstruating females: The current whole-blood-collection paradigm needs to be changed. Transfusion. 2006; 46(10):1667–1681. [PubMed: 17002622]
5. Sullivan JL. Stored iron and vascular reactivity. Arterioscler Thromb Vasc Biol. 2005; 25(8):1532–1535. [PubMed: 16055755]
6. Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, Malenka DJ, Ozaki CK, Lavori PW. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: results from a randomized trial. J Natl Cancer Inst. 2008; 100(14):996–1002. [PubMed: 18612130]
7. Lee PL, Halloran C, Trevino R, Felitti V, Beutler E. Human transferrin G277S mutation: a risk factor for iron deficiency anemia. Brit J Hem. 2001; 115(2):329–333.

8. Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and Iron-Overload Screening in a Racially Diverse Population. *N Engl J Med*. 2005; 352(17):1769–1778. [PubMed: 15858186]
9. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genetics*. 1996; 13(4):399–408. [PubMed: 8696333]
10. Cable RG, Glynn SA, Kiss JE, et al. Iron deficiency in blood donors: analysis of enrollment data from the REDS-II Donor Iron Status Evaluation (RISE) study. *Transfusion*. 2011; 51(3):511–522. [PubMed: 20804527]
11. American Association of Blood Banks. Standards for Blood Banks and Transfusion Services. 26th Edition. Bethesda MD: AABB; 2009. p. page 56Standard 5.4.1A,
12. Code of Federal Regulations. 21 CFR 640.3 (b) (3).
13. Melmed R, Cable RG, Wright DJ. Modeling Pre-Donation Venous Hemoglobin from Post-Donation Venous Hemoglobin. *Transfusion*. 2009; 49(3S):41A.
14. Punnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and its ratio to serum ferritin in diagnosis of irondeficiency anemia. *Blood*. 1997; 89(3):1052–1057. [PubMed: 9028338]
15. Punnonen K, Rajamaki A. Evaluation of iron status of Finnish blood donors using serum transferrin receptor. *Transfus Med*. 1999; 9(2):131–134. [PubMed: 10354382]
16. Radtke H, Meyer T, Ulrich K, et al. Rapid identification of iron deficiency in blood donors with red cell indexes provided by Advia 120. *Transfusion*. 2005; 45(1):5–10. [PubMed: 15647011]
17. Ali MA, Luxton AW, Walker WH. Serum ferritin concentration and bone marrow iron stores: a prospective study. *Can Med Assoc J*. 1978; 118(8):945–946. [PubMed: 647567]
18. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA. Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood*. 1990; 75(1):102–107. [PubMed: 2294984]
19. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood*. 1990; 75(9):1870–1876. [PubMed: 2331526]
20. Department of Health and Human Services. [Last accessed 3/23/2011] The 2007 National Blood Collection and Utilization Survey Report. Accessed at: <http://www.aabb.org/programs/biovigilance/nbcus/Documents/07nbcusrpt.pdf>.
21. Røsvik AS, Ulvik RJ, Wentzel-Larsen T, Hervig T. The effect of blood donation frequency on iron status. *Transfus Apher Sci*. 2009; 41(3):165–169. [PubMed: 19819187]
22. Bahrami, SH.; Guiltinan, AM.; Schlumpf, KS.; Scott, E.; Banks, LL.; D'Andrea, P.; Hartman, EL.; Vij, V.; Wright, DJ. Spencer B and Murphy EL for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II) Donation frequency of blood donors participating in a prospective cohort study of iron status. *Transfusion* epub: 7 JAN 2011, DOI: 10.1111/j.1537-2995.2010.03002.x
23. Mast A, Schlumpf K, Wright D, et al. Demographic Correlates of Low Hematocrit Deferral among Prospective Whole Blood Donors. *Transfusion*. 2010; 50(8):1794–1802. [PubMed: 20412525]
24. Pynaert I, De Bacquer D, Matthys C, et al. Determinants of ferritin and soluble transferrin receptors as iron status parameters in young adult women. *Public Health Nutrition*. 2009; 12(10): 1775–1782. [PubMed: 19105865]
25. Milman N, Kirchhoff M. Relationship between serum ferritin and risk factors for ischaemic heart disease in 2235 Danes, aged 30–60 years. *Journal Int Med*. 1999; 245(5):423–433.
26. Mast AE, Foster TM, Pinder HL, et al. Behavioral, biochemical, and genetic analysis of iron metabolism in highintensity blood donors. *Transfusion*. 2008; 48(10):2197–2204. [PubMed: 18657084]
27. [Last accessed 3/23/2011] National Center for Chronic Disease Prevention and Health Promotion, CDC. Accessed at: http://apps.nccd.cdc.gov/brfss/display_c.asp?yr_c=2009&yr=2002&cat=TU&state=US&bkey=20020014&qkey=4394&qtype=C&grp=0&SUBMIT2=Compare.
28. Nordenberg D, Yip R, Binkin NJ. The Effect of Cigarette Smoking on Hemoglobin Levels and Anemia Screening. *JAMA*. 1990; 264(12):1556–1559. [PubMed: 2395196]
29. Bianco C, Brittenham G, Gilcher RO, et al. Maintaining iron balance in women blood donors of childbearing age: summary of a workshop. *Transfusion*. 2002; 42(6):798–805. [PubMed: 12147035]

APPENDIX A

REDS-II DONOR IRON STATUS EVALUATION (RISE) STUDY BASELINE QUESTIONNAIRE

As part of the RISE study, sponsored by the National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH), we would like to ask you some questions about your blood donation history, smoking history, diet, use of vitamins and/or supplements, and for women, a few questions about your reproductive history. Your responses will help us better understand iron status in blood donors and contribute valuable information for improving the health of blood donors. Your answers to all questions will be kept confidential and only be used for the purpose of this research.

Today's Date: ____ ____ - ____ ____ - ____ ____
 Month Day Year

Blood Center ID: _____

Blood unit ID (BUI): _____

Sponsored by
National Heart, Lung, and Blood Institute
National Institutes of Health (NIH)

AFFIX LABEL WITH ID HERE

SECTION A**Your blood donation history:**

1. Is this the first time you have EVER donated blood? ☐ Yes {SKIP TO SECTION B, QUESTION 7} ☐ No	4. Please tell us the total number of blood donations you have made in the last 2 years. ____ ____ ____ WRITE THE NUMBER OF DONATIONS ☐ Don't Know
2. Including your most recent donation, how many times in your life have you donated blood? ☐ 1 to 2 times ☐ 3 to 5 times ☐ 6 to 10 times ☐ 11 to 20 times ☐ More than 20 times ☐ Don't Know	5. Were any of these donations made through a DIFFERENT blood center? ☐ Yes ☐ No ☐ Don't Know
3. Other than today, when was the last time you donated blood? ____ ____ ____ ____ ____ Month Year ☐ Don't Know	6. Were any of these apheresis donations? (Apheresis: Donors give only select blood components such as platelets, plasma, red cells, or a combination of these) ☐ Yes ☐ No

{IF YOUR LAST DONATION WAS MORE THAN 2 YEARS AGO SKIP TO SECTION B, QUESTION 7}

How many of these where aphaeresis donations?

NUMBER OF APHERESIS DONATIONS

SECTION B**Your smoking history:**

7. Have you smoked at least 100 cigarettes in your entire life? ☐ Yes ☐ No ☐ Don't know	9. Thinking about the last 30 DAYS (1 month), on how many of these days did you smoke? ____ ____ ____ WRITE THE NUMBER OF DAYS ☐ Don't know
8. Did you smoke ANY cigarettes during the last 90 DAYS (3 months)? ☐ Yes ☐ No {SKIP TO SECTION C QUESTION 11} ☐ Don't know	10. In the LAST 30 DAYS, on the days that you DID smoke, about how many cigarettes did you usually smoke per day? ____ ____ ____ WRITE THE NUMBER OF CIGARETTES ☐ Don't know

SECTION C**Your Diet:**

11. Over the LAST 12 MONTHS, about how many times per week did you eat the following foods?

[When thinking about the foods you eat, remember to include soups, stews, sandwiches, lunch meats, casseroles and salads that are made with these food items.]

	Never	Less than once/ week	Once/ week	Twice/week	3-4 times/ week	5-6 times/ week	Once every day	2 or more times/day
Liver (any kind)	☐	☐	☐	☐	☐	☐	☐	☐
Beef (including ground Beef)	☐	☐	☐	☐	☐	☐	☐	☐
Lamb, Pork, Chicken, Turkey	☐	☐	☐	☐	☐	☐	☐	☐
Clams	☐	☐	☐	☐	☐	☐	☐	☐
Oysters, Mussels, Shrimp, Sardines	☐	☐	☐	☐	☐	☐	☐	☐
Other Fish	☐	☐	☐	☐	☐	☐	☐	☐
Eggs	☐	☐	☐	☐	☐	☐	☐	☐
Dairy Products (Milk, Yogurt, Cheese)	☐	☐	☐	☐	☐	☐	☐	☐

SECTION D**Your use of vitamin pills, supplements and aspirin:**

12. Over the LAST 12 MONTHS, did you take any multivitamins such as One-A-Day, Theragran, or Centrum type multivitamins (as pills, liquids, or packets) on a regular basis (at least once a week)?

- ☐ Yes
☐ No
☐ Don't know

How often did you take multivitamins?

- ☐ Everyday
☐ 4 to 6 days per week
☐ 1 to 3 days per week
☐ Don't know

Does your multivitamin contain iron?

- ☐ Yes
☐ No
☐ Don't know

13. Over the LAST 12 MONTHS, did you take any iron supplements other than multivitamins on a regular basis (at least once a week)?

- ☐ Yes
☐ No
☐ Don't know

How often did you take iron supplements?

- ☐ Everyday
☐ 4 to 6 days per week
☐ 1 to 3 days per week
☐ Don't know

14. Do you currently take Aspirin or Aspirin containing pain relievers daily or nearly everyday?

☐ Yes
☐ No
☐ Don't Know

Why?

☐ For heart or cardiac health
☐ For pain relief
☐ For both

{MALE DONORS SKIP SECTION E AND GO TO END STATEMENT}

SECTION E
FOR FEMALE DONORS ONLY
Your reproductive history:

15. Which of these statements best describes your current menstrual status?

☐ I am still having periods and am NOT going through menopause
☐ I am still having periods, but am possibly going through menopause
☐ My periods have stopped completely because I have gone through menopause **{SKIP TO QUESTION 19}**
☐ I had an operation which stopped my periods **{SKIP TO QUESTION 19}**
☐ I am taking a medication that has stopped my periods completely **{SKIP TO QUESTION 19}**
☐ My periods have stopped because of other reasons **{SKIP TO QUESTION 19}**

16. What was the date when your last menstrual period started?

 Month Year

WRITE THE DATE OF YOUR LAST PERIOD

☐ I am having my period now

17. About how many periods did you have in the last year (12 Months)?

WRITE THE NUMBER OF PERIODS

18. How would you describe your menstrual flow or bleeding?

☐ **Spotting**, a drop or two of blood, not even requiring sanitary protection though you may prefer to use some.
☐ **Very light bleeding** (you would need to change the least absorbent tampon or pad one or two times per day, though you may prefer to change more frequently)
☐ **Light bleeding** (you would need to change a low or regular absorbency tampon or pad two or three times per day, though you may prefer to change more frequently)
☐ **Moderate bleeding** (you would need to change a regular absorbency tampon or pad every 3 to 4 hours, though you may prefer to change more frequently)
☐ **Heavy bleeding** (you would need to change a high absorbency tampon or pad every 3 to 4 hours, though you may prefer to change more frequently)
☐ **Very heavy bleeding or gushing** (protection hardly works at all; you would need to change the highest absorbency tampon or pad every hour or two)

The next few questions are about your pregnancy history. This information is very important to this study because it will help improve the health of all women. So please take whatever time you need to answer them as accurately and completely as possible.

19. Have you ever been pregnant? Please include live births, miscarriages, still births, tubal pregnancies and abortions.

☐ Yes
☐ No **{SKIP TO END STATEMENT}**
☐ Don't know

20. How many times have you been pregnant in your life? Again, be sure to include live births, miscarriages, still births, tubal pregnancies and abortions.

WRITE THE NUMBER OF PREGNANCIES
☐ Don't know

21. How many of your pregnancies resulted in a live birth? Please count the number of pregnancies, not number of live-born children. For example, if you had twins or other multiple births, count as a single pregnancy.

WRITE THE NUMBER OF PREGNANCIES
RESULTING IN LIVE BIRTHS
☐ No live births **{SKIP TO END STATEMENT}**

22. When was your last baby born?

Month Year

END STATEMENT

The survey is now complete. We appreciate you taking the time to complete this survey.
Your responses have provided us with valuable information.

Appendix B – Iron Status at RISE Enrollment by Donor Characteristics

	Number of Donors	Number and Percentage with AIS	Number and Percentage with IDE
All Donors	2425	365 (15.0)	1011 (41.7)
Red Cell Donations in 2 years prior to enrollment ^a			

	Number of Donors		Number and Percentage with AIS		Number and Percentage with IDE	
<i>p-value</i> ^b			<0.0001		<0.0001	
FT: 0 donations	303		17 (5.6)		49 (16.2)	
RA: 0 donations	585		14 (2.4)		80 (13.7)	
4 donations	483		84 (17.4)		258 (53.4)	
5–6 donations	370		95 (25.7)		215 (58.1)	
7–9 donations	464		104 (22.4)		271 (58.4)	
10+ donations	220		51 (23.2)		138 (62.7)	
Gender						
<i>p-value</i>			<0.0001		<0.0001	
Male	1175		126 (10.7)		384 (32.7)	
Female	1250		239 (19.1)		627 (50.2)	
Age (in years)	Male	Female	Male	Female	Male	Female
<i>p-value</i>			0.04		<0.0001	
20	63	70	1 (1.6)	15 (21.4)	10 (15.9)	39 (55.7)
20–29	164	179	13 (7.9)	43 (24.2)	38 (23.2)	89 (49.7)
30–39	165	175	17 (10.3)	32 (18.3)	49 (29.7)	88 (50.3)
40–49	215	282	19 (8.8)	54 (19.1)	57 (26.5)	149 (53.0)
50–59	306	320	48 (15.7)	62 (19.4)	125 (40.9)	151 (47.2)
60+	262	224	28 (10.7)	33 (14.7)	105 (40.1)	111 (49.6)
Race/Ethnicity ^c						
<i>p-value</i>			0.54		0.07	
White	2108		326 (15.5)		895 (42.5)	
Asian	76		8 (10.7)		21 (27.6)	
Black	117		13 (11.1)		48 (41.0)	
Hispanic	79		12 (15.2)		33 (41.8)	
Other	28		5 (17.9)		11 (39.3)	
Center						
<i>p-value</i>			0.14		0.02	
A	436		62 (14.2)		161 (36.9)	
B	390		60 (15.4)		161 (41.3)	
C	376		47 (12.5)		150 (39.9)	
D	392		57 (14.5)		155 (39.5)	
E	415		80 (19.3)		199 (48.0)	
F	416		59 (14.2)		185 (44.5)	
Weight ^c						
<i>p-value</i>			<0.0001		<0.0001	
<150	572		125 (21.9)		291 (50.9)	
150–174	595		89 (15.0)		253 (42.5)	
175–199	510		74 (14.5)		210 (41.2)	

	Number of Donors	Number and Percentage with AIS	Number and Percentage with IDE
200+	627	52 (8.3)	199 (31.7)
Smoking^c			
<i>p-value</i>		0.11	<0.0001
Current	297	38 (12.8)	93 (31.3)
Past	613	79 (12.9)	234 (38.2)
Never	1413	234 (16.6)	645 (45.7)
Iron Supplementation			
<i>p-value</i>		0.85	0.03
Takes supplemental iron	954	142 (14.9)	424 (44.4)
No supplemental iron	1471	223 (15.2)	587 (39.9)
HFE (C282Y, H63D Genotypes)^c			
<i>p-value</i>		0.81	0.17
Wild-Type	1568	238 (15.2)	667 (42.5)
Heterozygous – C282Y	194	34 (17.5)	86 (44.3)
Heterozygous – H63D	573	82 (14.3)	231 (40.3)
Two variant genes ^d	87	11 (12.6)	26 (29.9)
G277S Genotype^c			
<i>p-value</i>		0.99	0.55
Wild-Type	2107	318 (15.1)	876 (41.6)
Heterozygous	249	37 (14.9)	103 (41.4)
Homozygous	5	0 (0)	1 (20.0)
Menstrual Status (females only)^c			
<i>p-value</i>		0.12	0.13
Periods Stopped	566	98 (17.3)	272 (48.1)
Menstruating, not at enrollment	597	120 (20.1)	310 (51.9)
Menstruating at enrollment	68	19 (27.9)	39 (57.4)
Pregnancy Status (females only)^c			
<i>p-value</i>		0.96	0.49
Never Pregnant	405	80 (19.8)	192 (47.4)
Last pregnancy > 5 years ago	666	127 (19.1)	350 (52.6)
Pregnancy five years	90	15 (16.7)	44 (48.9)
No date of pregnancy given	76	14 (18.4)	35 (46.0)

^aRed cell donations is the number of whole blood and double red cell donations made in the 2 years prior to enrollment. Double red cells are counted as two donations.

^b*p*-value from the X² test

^cMissing values: Race/Ethnicity=18, Weight=121, Smoking=102, G277S=64, HFE=3, Menstrual=19, Pregnancy=13

^dHomozygous for C282Y or H63D or mixed heterozygous

Figure 1. Adjusted odds ratios and 95% confidence intervals for hemoglobin deferral overall and for females alone by weeks since last red cell donation

ORs are based on reference group of donation intervals ≥ 26 weeks. Squares correspond to mean ORs for all return donations; Bars represent 95% CI. Diamonds represent mean ORs for female donations (no 95% CI presented). There were insufficient male deferrals to analyze by week.

Figure 2. Adjusted odds ratios and 95% confidence intervals for absent iron stores (AIS) overall and for females alone by weeks since last red cell donation

ORs are based on reference group of donation intervals ≥ 26 weeks. Squares correspond to mean ORs for all donations; Bars represent 95% CI. Diamonds represent mean ORs for female donations (no 95% CI presented). There were insufficient male observations with AIS to analyze by week.

Panel A: Proportion of donors with absent iron stores (AIS)

Panel B: Proportion of donors with iron deficient erythropoiesis (IDE)

Panel C: Proportion of donors with venous hemoglobin < 12.5 g/dL **Figure 3.**

Panels A–C: The proportion of donors with indicated laboratory results at enrollment and final visits, by gender and donation category. Donors are accepted for donation based on fingerstick hemoglobin measurements which are not always concordant with venous hemoglobin. There were 1,334 enrolled RISE donors for whom data at enrollment and at the final visit were available.

Panel A: Proportion of donors with absent iron stores (AIS, defined by ferritin < 12ng/mL).

Panel B: Proportion of donors with iron deficient erythropoiesis (IDE, defined as $\log[sTfR/\text{ferritin}] \geq 2.07$).

Panel C: Proportion of donors with venous hemoglobin < 12.5 g/dL.

Table 1

Donor enrollment, numbers of donors with return visits/final visits, average time in study, average number of red cell donations, hemoglobin deferrals after enrollment and mean donation interval by the four cohorts

	First time/Reactivated Donors		Frequent Donors	
Gender	FEMALES	MALES	FEMALES	MALES
Enrolled at Baseline	481	407	769	768
Number of visits after enrollment				
Did not return	102	119	30	19
1 (# with final visit)	104 (10)	82 (10)	57 (4)	43 (1)
2-3 (# with final visit)	142 (75)	98 (50)	149 (64)	116 (39)
4-5 (# with final visit)	68 (49)	68 (54)	185 (125)	235 (164)
6+ (# with final visit)	65 (53)	40 (35)	348 (293)	355 (308)
Total Donors Returned (# with final visit)	379 (187)	288 (149)	739 (486)	749 (512)
Average time in study	429 days	440 days	516 days	524 days
Average return red cell donations per donor	2.6	2.9	4.4	5.2
Average Hemoglobin deferrals per donor	0.55	0.06	0.79	0.18
Mean days since previous donation (for <u>all</u> return visits)	156 days	146 days	113 days	101 days

Table 2Characteristics of All Return Visits and Return Visits with Hemoglobin Deferral^a

	Return Visits ^b	Hemoglobin/Hematocrit Deferral Visits	Deferral Rate %
	N		
Total	9901	945	9.5%
Donor Gender and Age			
Female	5047	793	15.7
Male	4854	152	3.1
Female			
<30	541	112	20.7
30–39	543	100	18.4
40–49	1164	204	17.5
50–59	1562	229	14.7
60+	1237	148	12.0
Male			
<30	438	8	1.8
30–39	513	8	1.6
40–49	896	15	1.7
50–59	1459	53	3.6
60+	1548	68	4.4
Blood Center			
center A	2044	181	8.9
center B	1620	208	12.8
center C	1427	135	9.5
center D	1439	129	9.0
center E	1667	183	11.0
center F	1704	109	6.4
Number of Donations in Previous 24 months			
1–3 donations	1852	227	12.3
4–6 donations	2741	309	11.3
7–9 donations	3451	313	9.1
10+ donations	1857	96	5.1

^aOnly accepted donors were enrolled. Thus, enrollment visits cannot be used to assess hemoglobin deferral.^bNot including 268 other deferrals and 101 sample only visits.

Table 3

Prevalence of iron deficiency among visits with available ferritin and sTfR results.

	Tested Visits ^a		Tested Visits with AIS ^b		Tested Visits with IDE ^b	
	N	%	N	%	N	%
Tested Visits	6449	1421	22.0	3228	50.1	
Donor Gender and Age						
Female	3938	1094	27.8	2373	60.3	
Male	2511	327	13.0	855	34.1	
Female						
<30	529	175	33.1	330	62.4	
30-39	456	139	30.5	288	63.2	
40-49	944	274	29.0	601	63.7	
50-59	1144	324	28.3	666	58.2	
60+	865	182	20.3	488	56.4	
Male						
<30	421	28	6.7	94	22.3	
30-39	318	26	3.7	84	26.4	
40-49	452	43	9.5	118	26.1	
50-59	691	133	19.2	296	42.8	
60+	629	97	15.4	263	41.8	
Blood Center						
center A	1625	364	22.4	824	50.7	
center B	877	167	19.0	403	46.0	
center C	533	80	15.0	224	42.0	
center D	1045	221	21.1	519	49.7	
center E	1413	409	28.9	813	57.5	
center F	956	180	18.8	445	46.5	
Number of Donations in Previous 24 months						
Zero (FT/RA enrollment visit)	906	33	3.6	135	14.9	

	Tested Visits ^a		Tested Visits with AIS ^b		Tested Visits with IDE ^b	
	N	N	%	N	%	%
1–3 donations	1606	212	13.2	605	37.7	
–6 donations	1749	489	28.0	1036	59.2	
7–9 donations	1475	466	31.6	988	67.0	
10+ donations	713	221	31.0	464	65.1	

^a 6449 visits were tested out of a total 12,695 visits. Visits selected for testing were based on a higher likelihood of iron depletion (see Methods).

^b AIS: absent iron stores; IDE: iron deficient erythropoiesis

Table 4Adjusted odds ratios^a for hemoglobin deferral, absent iron stores (AIS) and iron deficient erythropoiesis (IDE)

	Hemoglobin Deferral Model OR (95% CI)		AIS model OR (95% CI)		IDE model OR (95% CI)	
Race/Ethnicity^b						
<i>p-value</i>	0.05		0.89		0.03	
Asian vs. White	1.2 (0.7–2.3)		0.9 (0.5–1.8)		0.6 (0.4–0.9)	
Black vs. White	2.1 (1.3–3.5)		1.1 (0.6–2.0)		1.6 (1.0–2.5)	
Hispanic vs. White	1.6 (1.0–2.7)		1.1 (0.6–1.9)		1.0 (0.6–1.6)	
Other vs. White	1.7 (0.9–3.5)		1.5 (0.7–3.4)		0.9 (0.4–1.8)	
Gender^c						
<i>p-value</i>	<0.0001		<0.0001		<0.0001	
Female vs. Male	6.3 (3.8–10.5)		2.9 (1.9–4.7)		3.0 (2.1–4.2)	
Age (in years)						
<i>p-value</i>	0.01		0.18		0.006	
< 30 vs. 40–49	0.9 (0.2–3.2)		1.3 (0.7–2.3)		1.8 (1.2–2.7)	
30–39 vs. 40–49	0.9 (0.3–2.9)		1.1 (0.6–1.9)		1.7 (1.2–2.6)	
50–59 vs. 40–49	1.9 (0.9–4.0)		1.6 (1.0–2.5)		1.8 (1.3–2.5)	
60+ vs. 40–49	1.6 (0.8–3.5)		1.1 (0.7–1.8)		1.4 (0.9–2.0)	
Weight						
<i>p-value</i>	0.38		0.009		0.0008	
<150 vs. 150–174	0.9 (0.5–1.7)		1.0 (0.7–1.5)		1.0 (0.7–1.3)	
175–199 vs. 150–174	1.7 (0.6–5.2)		0.6 (0.2–1.7)		0.9 (0.5–1.7)	
200+ vs. 150–174	0.7 (0.4–1.2)		0.6 (0.4–0.9)		0.6 (0.4–0.8)	
Smoking						
<i>p-value</i>	0.53		0.006		<0.0001	
Current vs. Never	0.8 (0.6–1.2)		0.6 (0.4–0.9)		0.5 (0.4–0.7)	
Past vs. Never	1.1 (0.9–1.3)		0.8 (0.6–1.0)		0.6 (0.5–0.8)	
Iron Supplementation						

	Hemoglobin Deferral Model OR (95% CI)	AIS model OR (95% CI)	IDE model OR (95% CI)
<i>p</i> -value	0.69	<0.0001	<0.0001
Takes supplemental iron vs. None	1.0 (0.8–1.3)	0.6 (0.5–0.7)	0.7 (0.6–0.8)
HFE (C282Y, H63D)			
<i>p</i> -value	0.90	0.75	0.02
Heterozygous C282Y vs. WT	1.0 (0.7–1.4)	0.6 (0.4–0.9)	0.9 (0.7–1.2)
Heterozygous H63D vs. WT	1.0 (0.8–1.2)	0.8 (0.6–1.0)	0.9 (0.7–1.1)
Homozygous for C282Y or H63D or mixed heterozygote vs. WT ^d	1.2 (0.8–1.9)	0.8 (0.4–1.3)	0.5 (0.3–0.7)
Red Cell Donations in previous 2 years			
<i>p</i> -value	0.89	<0.0001	<0.0001
<4 donations vs. reference ^e	Reference	2.5 (1.6–3.9)	2.2 (1.6–2.9)
4–6 donations vs. reference ^e	0.9 (0.7–1.3)	9.2 (5.6–15.0)	9.3 (6.5–13.3)
7–9 donations vs. reference ^e	1.0 (0.8–1.3)	6.4 (4.0–10.1)	5.6 (4.0–7.7)
10+ donations vs. reference ^e	1.0 (0.8–1.4)	8.6 (5.3–14.0)	8.2 (5.8–11.5)
Center			
<i>p</i> -value	0.0007	0.007	0.03
A vs. F	1.2 (0.8–1.7)	1.0 (0.8–1.5)	0.8 (0.6–1.1)
B vs. F	2.0 (1.4–2.9)	0.8 (0.6–1.2)	0.8 (0.6–1.1)
C vs. F	1.3 (0.9–1.9)	0.9 (0.6–1.3)	0.9 (0.7–1.2)
D vs. F	1.5 (1.0–2.3)	1.2 (0.8–1.7)	0.9 (0.7–1.2)
E vs. F	1.7 (1.2–2.5)	1.6 (1.1–2.2)	1.3 (1.0–1.6)
Time Since last donation			
<i>p</i> -value	<0.0001	<0.0001	<0.0001
M	See Figure 1	See Figure 2	See text

^a Adjusted for all listed variables, as well as for last red cell donation type (single or double), presence of the G277S polymorphism, pregnancy history/time since last pregnancy and menstrual status.

^b Race/ethnicity was self-reported by REDS-II blood donors on questionnaires routinely administered at the blood centers. Separate questions were used to determine Hispanic ethnicity and race; for analysis these questions were combined, with Hispanic ethnicity taking precedence over racial identification for the purposes of the variable.

^c The standardized gender effect (i.e. applies the overall age and weight distributions to both genders).

^dInsufficient numbers of the following individual genotypes for the model: C282Y/C282Y (7); H63D/H63D (39); and C282Y/H63D (41), thus all three genotypes were combined.

^eReference group is 1–3 donations for hemoglobin deferral model and 0 donations for the AIS and IDE models.