Published in final edited form as:

Transfusion. 2011 March ; 51(3): 511–522. doi:10.1111/j.1537-2995.2010.02865.x.

Iron Deficiency in Blood Donors: Analysis of Enrollment Data from 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, Vibha Vij⁶, and Toby L. Simon⁹ for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II)

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

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

³ Institute for Transfusion Medicine, Pittsburgh, PA

⁴ Blood Center of Wisconsin, Milwaukee, WI

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

⁶ Westat, Rockville, MD

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

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

⁹ CSL Plasma, Boca Raton, FL

Abstract

Background—Regular blood donors are at risk of iron deficiency, but characteristics which predispose to this condition are poorly defined.

Methods—2425 red cell donors, either first time (FT) or reactivated donors (no donations for 2 years) or frequent donors were recruited for follow-up. At enrollment, ferritin, soluble transferrin receptor (sTfR), and hemoglobin were determined. Donor variables included demographics, smoking, dietary intake, use of iron supplements, and menstrual/pregnancy history. Models to predict two measures of iron deficiency were developed: Absent iron stores (AIS) were indicated by ferritin < 12 ng/mL and iron deficient erythropoiesis (IDE) by log (sTfR/ferritin) ≥ 2.07.

Results—15.0% of donors had AIS, 41.7% IDE. In frequent donors, 16.4% and 48.7% of males had AIS and IDE, respectively, with corresponding proportions of 27.1% and 66.1% for females. Donation intensity was most closely associated with AIS/IDE (ORs from 5.3 to 52.2 for different donation intensity compared to FT donors). Being female, younger, and/or menstruating also increased the likelihood of having AIS/IDE, as did having a lower weight. Marginally significant

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.

Reprints will not be available from the author.

Alan Mast gives educational talks about anemia, including iron deficiency anemia, for Siemens Corporations. He has never received over \$10,000 in one year.

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

variables for AIS and/or IDE were being a non-smoker, previous pregnancy and not taking iron supplements. Dietary variables were in general unrelated to AIS/IDE, as was race/ethnicity.

Conclusion—A large proportion of both female and male frequent blood donors have iron depletion. Donation intensity, gender/menstrual status, weight, and age are important independent predictors of AIS/IDE. Reducing the frequency of blood donation is likely to reduce the prevalence of iron deficiency among blood donors, as might implementing routine iron supplementation.

Keywords

Donors; Hematology—Red Cells; Blood Center Operations

Introduction

Whole blood donation, which can be as frequent as every eight weeks in the United States, is associated with depletion of body iron stores.^{1,2} Studies by Finch³ and Simon¹ in the late 1970's and 1980's showed that there was a drop in serum ferritin concentration associated with blood donation over the prior 4–5 years. The over-all prevalence of iron depletion was 8% in male blood donors and 23% in female blood donors in Simon's studies indicating the impact of menstrual blood loss. Simon's studies showed this problem could be ameliorated by iron supplementation.

Since those studies 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. In addition, the use of intensive donor recruitment strategies and double red cell collections have probably also contributed to more frequent blood donation. These factors have likely increased the problem of iron depletion.

A polymorphism in transferrin, which increases the likelihood that menstruating women will be iron deficient,⁴ and description of several genes for hemochromatosis, at least one of which (C282Y) is associated with iron absorption,^{5,6} have been described. This leads to the possibility these genetic markers might define “at risk” and “protected” donor groups with respect to iron depletion in blood donors. Further, none of the previous studies of iron depletion in blood donors have assessed the influence of racial or socio-economic factors. Dietary intake would also be expected to have an important influence on the prevalence of iron depletion in various donor groups, and has not been studied. Finally, the availability of new tests of body iron status provides further opportunities to detect or prevent this problem.

In addition to causing anemia, iron deficiency has been tentatively linked to a variety of neurologic and other non-hematologic manifestations,⁷ but may also be cardio-protective.⁸ Thus, significant changes in blood donor management to prevent iron depletion in blood donors⁹ should be considered in the context of careful evaluation of the pros and cons of being iron depleted, which may depend in part on varying degrees of iron depletion.⁸

Underlying any plan to modify the iron status of blood donors is the requirement for a more precise understanding of the factors that predict blood donor iron deficiency. For these reasons, the National Heart, Lung, and Blood Institute's (NHLBI) Retrovirus Epidemiology Donor Study – II (REDS-II) program launched the REDS-II Donor Iron Status Evaluation (RISE). The RISE study aims to evaluate the effects of blood donation intensity on iron and hemoglobin status, assess factors that could modify that relationship, and provide data to help formulate optimal whole blood donation frequency guidelines. In this manuscript we report the analysis of the RISE enrollment data. This large and comprehensive study provides an updated evaluation of iron status in the US blood donor population.

Methods

The RISE study was conducted between December 2007 and December 2009 as a twenty four month longitudinal multi-center study. All six REDS-II blood centers participated in the study; these included the American Red Cross New England Region (Dedham, MA), American Red Cross Southern Region (Douglasville, GA), Blood Center of Wisconsin (Milwaukee, WI); Blood Centers of the Pacific (San Francisco, CA), Hoxworth Blood Center/University of Cincinnati Academic Health Center (Cincinnati, OH), and the Institute for Transfusion Medicine (Pittsburgh, PA). The REDS-II centers represent geographically and demographically diverse populations and collectively account for over 8% of annual blood collections in the United States. The REDS-II coordinating center is Westat (Rockville, MD) and Blood Systems Research Institute (San Francisco, CA) serves as the REDS-II Central Laboratory.

Enrollment

Two cohorts were established and followed for a maximum of 24 months. The first time/reactivated (FT/RA) donor cohort 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 a frequent donor cohort consisting of men who had given ≥ 3 whole blood donations in the last year and women who had given ≥ 2 whole blood donations in the last year – or equivalent double red cell donations. Whole blood donation containers at the time of enrollment were for 500 mL whole blood draw at 5 of the blood centers and for 450 mL draw at the other blood center.

The target enrollment goal for the RISE study was 2,340 donors – 1,500 frequent donors and 840 first time/reactivated donors. Enrollment procedures were targeted to recruit equal number of male and female donors in each cohort. Enrollment in the study mainly occurred at fixed donation sites. However, some study sites chose to recruit from a combination of fixed and mobile sites to meet their targets. Based on the study inclusion criteria, only those individuals who presented to donate whole blood or double red blood cell units and were not deferred were approached to participate in the RISE study.

Donors agreeing to enroll in the study reviewed and signed an informed consent, agreed to return to donate frequently in the next 24 months, provide blood samples at each visit, and complete a baseline questionnaire. The RISE study protocol and donor consent forms were approved by each participating center IRB and the Westat IRB.

Donor Questionnaire/Donor Demographics

A self-administered questionnaire was used to collect details on blood donation history; smoking history; diet; use of vitamins, supplements and aspirin; and reproductive history (female donors only). Most of the questions were adopted from previously validated instruments [California Smoking Survey,¹⁰ NIH Diet History Questionnaire,¹¹ NHANES Reproductive Questionnaire 2000¹² Mansfield et al.¹³]. Some questions were condensed for analysis purposes. RISE donors enrolling in the study were asked about their average consumption of certain iron rich foods in the past 12 months. The donors could answer in nine dietary frequency levels, which were regrouped into 3 levels based on each food's frequency approximating a low, medium and high level of consumption for each food. The exception was liver which was dichotomized because the majority reported never eating liver. A history of iron supplementation was determined by asking the donor if they took a multivitamin with iron and/or if they took a specific iron supplement. Height, weight, country of birth, race/ethnicity, and highest educational level were compiled from the donor information form that donors complete at the time of donation at the REDS-II centers. See

Appendix A for the complete RISE enrollment questionnaire. The survey instrument (i.e. questionnaire) was approved by the U.S. Office of Management and Budget.

Laboratory Testing at the Enrollment Visit

Plasma Ferritin and Soluble Transferrin Receptor (sTfR)—Body iron levels were measured by testing for plasma ferritin and sTfR. Ferritin was measured using the ADVIA Centaur Ferritin Assay, an immunoassay using direct chemiluminometric technology and a constant amount of two anti-ferritin antibodies (Siemens Healthcare Diagnostic Inc., Deerfield, IL). A particle-enhanced immunoturbidimetric assay was used to detect sTfR, in which latex bound anti-sTfR antibodies react with the antigen in the sample to form an antigen/antibody complex that is measured turbidimetrically (Tina-quant Soluble Transferrin Receptor Assay, Roche Diagnostics, Indianapolis, IN).

Fingerstick hemoglobin/hematocrit and venous Hemoglobin testing—Prior to donation and enrollment, quantitative fingerstick hemoglobin/hematocrit levels were measured using each center's routine operational methods. Copper sulfate screening was not used. All centers also measured venous hemoglobin on either a pre-donation or post-donation EDTA sample using the HemoCue® Hb 201 analyzer (HemoCue, Inc., Lakeforest, CA). Post donation venous hemoglobin values (Post vHb) representing approximately 24% of all samples were converted to estimated pre-donation values (Pre vHb) using the formula $\text{Pre vHb(g/dL)} = \text{Post vHb} + 0.8423 - (0.002035 \times \text{Weight (lbs)})$. The formula was developed based on simultaneous measurement of pre- and post- donation venous hemoglobin determined in 278 whole blood donors.¹⁴

HFE and Transferrin Gene Mutation Testing—Samples were screened for the C282Y and H63D mutations in the HFE gene which predispose to hemochromatosis,⁶ and for the C277Y mutation on the transferrin gene which predisposes to iron deficiency anemia⁴ using DNA isolated from frozen whole blood samples and SyBr green based real-time polymerase chain reaction (PCR).

Determination of Absent Iron Stores (AIS) and Iron Deficiency Erythropoiesis (IDE)

A subject was classified as having absent iron stores (AIS) if their plasma ferritin at the enrollment visit 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.^{15, 16, 17, 18} Measurement of the soluble transferrin receptor (sTfR) is a sensitive measure of functional iron deficiency.^{19,20} In addition, ferritin measurements and sTfR values have been combined into a ratio, $\log(\text{sTfR/ferritin})$, as a derived measurement. Use of the two reciprocally regulated measurements appears to provide excellent discrimination of clinical iron deficiency anemia and early experience in blood donors suggests high sensitivity in the detection of iron deficient erythropoiesis.^{15, 17} Here, iron deficient erythropoiesis (IDE) is defined as present if the log of the ratio of soluble transferrin receptor (sTfR) to ferritin [$\log(\text{sTfR/Ferritin})$] was ≥ 2.07 . This value corresponded to the 97.5 percentile of the distribution of the $\log(\text{sTfR/ferritin})$ in FT/RA males at enrollment. Other studies have used higher cut-offs based on a gender-neutral reference range in first time donors.¹⁷ Given the significant differences between men and women in iron stores, we favored male first time donors as the reference population.

Statistical Analysis

The arithmetic means of hemoglobin and the geometric means of ferritin were calculated (geometric rather than arithmetic means were used because the ferritin distributions were heavily skewed to the right) by gender, FT/RA versus frequent status, and by number of

donations in the year prior to enrollment. Bivariable associations between AIS or IDE and all donor characteristics were evaluated using chi-square statistics. Multivariable logistic regression models for AIS and IDE incorporating all potential predictor variables were constructed to evaluate independent risk factors of AIS and IDE (SAS 9.1.3 (2004) SAS Institute Inc, Cary NC). The study was designed to prospectively follow cohorts; this cross-sectional analysis of the cohorts was not statistically powered to consider most of the potential variables associated with AIS or IDE. Hence, all potential predictor variables were retained in the multivariable logistic regressions so that plausible magnitude of associations could be noted. A more parsimonious model containing only statistical significant (at 0.05 level) variables yielded substantively equivalent odds ratios for the remaining variables compared to the model presented. An age by gender interaction was included in each regression; and the gender effect described in the model summary compares male donors averaged over the observed male age distribution to female donors averaged over the observed female age, menstrual, and pregnancy distributions.

Results

The final enrollment in RISE was 2425 donors: 481 FT/RA females; 407 FT/RA males; 769 frequent female donors and 768 frequent male donors. Table 1 shows the four main indicators of iron status, venous hemoglobin, ferritin, sTfR, and log (sTfR/Ferritin), by gender and donor status at enrollment. Median hemoglobin levels were higher in males than in females. However frequent male donors had lower median hemoglobin compared to FT/RA males. Additionally, FT/RA male donors had a median ferritin of 108 ng/mL while frequent male donors had a median ferritin level of 25 ng/mL; corresponding levels for female donors were 37 ng/mL (FT/RA) and 19 ng/mL (frequent). The median log (sTfR/Ferritin) was also higher in frequent than in FT/RA donors of the same sex.

Of the 2425 donors in the RISE study, 15.0% had AIS and 41.7% had IDE at enrollment. For FT/RA donors 0% of males had AIS and 2.5% (by definition) had IDE at enrollment, whereas 6.4% of FT/RA females had AIS and 24.7% IDE. In contrast, 16.4% and 48.7% of frequent male donors had AIS and IDE, respectively, with corresponding proportions of 27.1% (AIS) and 66.1% (IDE) for frequent female donors (Table 1).

Figure 1 shows that venous hemoglobin decreased, albeit slightly, for males with increasing numbers of donations over the previous 12 months. Figure 2, representing the geometric mean of ferritin stratified by gender and number of donations in the last 12 months, shows that the difference between male and female frequent donors is small compared to the difference between frequent donors and donors with no donations in the last 12 months. Ferritin decreases with increasing donation frequency and this decrease was more marked in men: ferritin in males with >5 annual donations was nearly as low as the level observed in comparable females from both the pre- and post-menopausal age groups.

Table 2 shows the proportion of donors with AIS and IDE by demographics (Panel A), dietary intake (panel B), weight, smoking history, selected medications (aspirin and iron supplements), HFE and transferrin gene mutation status, and for females, menstrual and pregnancy history (panel C). Female donors had higher proportions of AIS (19.1%) and IDE (50.2%) than did male donors (10.7% and 32.7%, respectively). Reactivated donors had the lowest percentage of AIS (2.4%) and IDE (13.7%) followed closely by first-time donors, 5.6% and 16.2%, respectively. The percentage of donors with AIS increased to 17.4% for donors who had given 4 or fewer donations in the past 2 years and then remained in the low to mid 20% range for other donation frequencies. The percentage of donors with IDE increased monotonically with increasing numbers of previous donations in the past two years. Other demographic factors that had significant bivariable association with AIS and/or

IDE included age and country of birth. Race/ethnicity was not statistically significant overall, although Asians appeared to have a lower prevalence of IDE ($p=0.03$) compared to Whites. AIS was associated with heavier dairy consumption, but no other dietary variables were significant. For IDE, beef; dairy; liver; oysters, mussels, shrimp and sardines; and other fish consumption had p values less than 0.05 with higher consumption of beef and other fish seemingly protective for IDE (Table 2, Panel B). Lower weight, not smoking and iron supplementation were the only variables in Table 2, Panel C significantly associated with AIS and/or IDE.

All the donor characteristic variables in Table 2 were retained in the multivariable regressions for AIS and IDE. The results of these regressions are shown in Table 3 and in Appendix B. Table 3 includes variables with p -values less than or equal to 0.01 or observed Odds Ratios with at least twofold variation among dominant categories of the variable (i.e. greater than 5% of donors). Remaining variables included in the model but not meeting the above criteria are summarized in Appendix B.

The strongest predictor of having AIS or IDE at enrollment into the RISE study was the number of red cell donations in the past two years. Frequent donors with four or fewer prior donations in the past two years had 5.3 times the odds of a first-time donor having AIS, while donors with 7–9 donations had 13.5 times the odds (Table 3). Each increase in the number of donations resulted in significantly higher odds of having AIS. Because reactivated donors appeared to have a lower likelihood of having AIS than first-time donors based on the proportion of donors with AIS and IDE, they were modeled as separate groups. However when controlling for other factors in the adjusted model, the odds ratio comparing the odds of AIS in reactivated donors to those of first-time donors was not significant. Similar to AIS, red cell donation in the previous 2 years was the primary predictor of IDE. Frequent donors with four or fewer previous donations had 14.0 times the odds of IDE than that of a first-time donor and those with 10 or more donations in the last 2 years had 50.5 times the odds.

Female donors were more likely to have AIS and IDE than male donors. The OR was 1.8 comparing the odds of AIS in females to that of males and 2.8 for IDE when the gender effect was standardized for age, menstrual status, and pregnancy history. Age was a significant predictor of AIS, but only in females (Table 3). Female donors less than 20 years old had an odds ratio of 3.1 and 20–29 year olds 3.9 times that of 40–49 year olds. For IDE, all the younger female donor groups had a significantly higher odds of IDE compared to the 40–49 year olds. Male donors aged 20–29 had an odds ratio of 2.3 for IDE compared to the reference group. There were no other significant odds ratios for men. Center of enrollment was also a significant predictor of AIS ($p=0.01$) and IDE ($p<0.0001$) in the adjusted models. Center E was the only center with a significant odds ratio for AIS and IDE with center E donors being about 2 times more likely than donors at center A to have AIS or IDE.

Weight was significant for both AIS and IDE although less so for IDE ($p=0.0001$ vs. $p=0.04$). As body weight increased, the odds of AIS decreased, with persons over 200 pounds having the lowest odds ratio (OR: 0.5) compared to the reference group of donors weighing 150–174 pounds. Donors over 200 pounds also had significantly lower odds of IDE than donors who weighed 150–174 pounds (OR: 0.7, 95% CI: 0.5–0.9). Certain behaviors also impacted the likelihood of AIS or IDE. Taking iron supplements appeared to have a protective effect for AIS (OR: 0.7) but not for IDE. Conversely current (OR of 0.5) and past smoking (OR of 0.7) reduced the likelihood of IDE but not the likelihood of AIS. Donors with 2 HFE genes (homozygotes for C282Y or H63D or mixed heterozygotes) have a lower prevalence of IDE than donors who were wild-type for both genes (OR: 0.4), but having only one copy of either mutation was not protective. After adjusting for the

combined age/gender variable there was a residual effect of current menstrual status which increased the likelihood of IDE compared to the reference group but the effect was only marginal ($p=0.05$). Finally, women who had been pregnant had higher odds of IDE than women (and men) who had never been pregnant (Table 3).

Discussion

The RISE study was designed, in part, to evaluate the effects of blood donation intensity on iron and hemoglobin status and assess how these are modified as a function of donor demographic, reproductive and behavioral factors, and to provide data to help formulate optimal whole blood donation frequency guidelines. The analysis of the enrollment data from RISE presented here provides the largest and most comprehensive study to date of blood donor iron deficiency.

There have been three previous large cross-sectional studies that evaluated blood donor iron status. Finch, et al.,³ used the newly available ferritin assay in 1977 to study blood donors. Ferritin was significantly lower in frequent than in first time donors, and the proportion of donors with ferritin <12 ng/mL increased as the number of donations in the previous year increased. Simon, et al. performed a similar study, but with additional information on female donor menstrual status and on use of over-the-counter iron supplements.¹ Ferritin values at any donation frequency were higher in non-menstruating compared with menstruating women; and among menstruating women, in those taking self-administered iron supplements. Most recently Radtke, et al. studied German blood donors in a similar manner,¹⁷ but also evaluated sTfR and a number of red cell and reticulocyte indices. Using ferritin <12 ng/mL as an indicator of reduced iron stores, their findings were similar to those of Finch.

Analysis of the enrollment data from the RISE study shows that absent iron stores – AIS, and iron deficient erythropoiesis – IDE, are highly prevalent in frequent blood donors. Of RISE frequent male blood donors 16.4% have AIS and 48.7% IDE, while 27.1% and 66.1% of female frequent donors have AIS and IDE, respectively. In contrast to previous studies, two distinctly different donor groups were included in the RISE study. A first group consisted of first time and reactivated donors. We found no meaningful differences between FT and RA donors when controlling for other factors and the two groups were combined for most analyses. These donors were assumed to have an iron status that was unaffected by blood donation preceding enrollment. In the second group, we enrolled frequent whole blood and red cell donors who had a wide range of donation intensities measured over the previous 2 years. The RISE enrollment results are similar to the previous three studies in that donors' gender and previous donations were the most important factors in determining iron status. In this study we chose to use a specific measure of iron loss, reflected by ferritin <12 ng/mL (AIS), and a more sensitive measure of iron loss than previous study,¹⁷ $\log$ (sTfR/ferritin) >2.07 (IDE). As expected, our more sensitive definition resulted in a higher percentage of blood donors found to have IDE.

Deferral for low hemoglobin is the most common cause of presenting donor loss, particularly in females. Based on data from the REDS-II donor centers, 9.9% of all donation attempts (17.7% females; 1.6% males) end in a deferral for a low hemoglobin or hematocrit.²¹ Accepted blood donors are required to have a hemoglobin value of at least 12.5 g/dL in the United States.^{22,23} However, based on currently accepted hemoglobin reference ranges for all adults,²⁴ some female blood donors with values within the reference range are deferred, while some male donors who are below the reference range are eligible to donate. An analysis of NHANES II data for adults shown to have adequate iron stores²⁵ also shows that 12.5 g/dL is not an appropriate lower limit for blood donation to prevent iron depletion.

We confirmed that there are only minimal changes in donor hemoglobin in frequent donors as a function of prior donation intensity, replicating Simon, et al.¹ The results, however, are likely directly influenced by the cross-sectional nature of the study designs, since donors who became anemic as a result of iron depletion would have been expected to be deferred from previous blood donation, and hence not included in the group of frequent donors. Given the high rate of hemoglobin deferrals in the 6 REDS-II blood centers,²¹ this culling effect of deferred donors is likely to be significant. Our study also was limited in that we only enrolled donors who passed the operational requirements at each blood center. Thus, the prevalence and degree of anemia in presenting blood donors was not assessed. For these reasons, this study is unable to provide any information regarding the value of hemoglobin screening to detect iron deficiency or recommend any specific hemoglobin cut-off. Other studies, however, have addressed this issue and concluded that the hemoglobin screen is a poor assessment of the iron status of blood donors.^{17, 26} We expect to be able to address these hemoglobin cut-off issues in our follow-up study of these cohorts in RISE.

In our multivariable models for AIS and IDE, previous donation intensity was by far the most important predictor of iron deficiency and iron deficient erythropoiesis at enrollment. The magnitude of the associations between AIS or IDE and a variety of donor characteristics is a new and significant additional contribution of RISE. The following donor characteristics, in addition to donation intensity and gender, were found to be independent predictors of AIS and/or IDE: age, weight, smoking, use of iron supplements, HFE genotype, and menstrual and pregnancy status. Country of birth and one dietary variable, consumption of other fish, were marginally significant for IDE.

The inclusion of age, along with a separate assessments of menstrual and pregnancy status in the model, tended to blunt the observed gender effect in this analysis. The observed odds ratio for female gender, reflects a residual gender effect after adjustment for age and menstrual and pregnancy status, and probably reflects the residual iron depletion found in older, non-menstruating women compared to men. The independent influence of menses and pregnancy presumably reflects their known synergistic effect of lowering iron stores. Asian donors had a lower prevalence of IDE and were less likely to have IDE than whites in the unadjusted model. This association was not seen in the multivariable model and it is not clear if the latter finding relates to the country of birth differences or if the unadjusted ethnicity relationship might be due to genetic differences or to environment factors, for instance diet. One large report describes higher ferritin levels in Asians than in Caucasians, which is unrelated to HFE genotype.⁵ Presumably, this could explain the differences we observed.

The influence of 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 and since approximately 85% of body iron is found in the erythron. The independent effect of a tendency to lower weight in female donors tends to aggravate the existing female disadvantage in iron homeostasis.

Smoking had an apparent protective effect of blood donors' iron status. This was somewhat unexpected since smoking is known to increase hemoglobin levels.²⁷ It was expected that the higher hemoglobin level might allow smokers to donate more frequently at lower iron levels. However, one cross-sectional study in 788 women of menstrual age found that smoking lowers the sTfR, but not the ferritin after adjusting for all other measured variables.²⁸ This finding, if confirmed, could relate to our observation of higher odds of IDE, but not AIS in non-smokers. It is not clear from the cited study if this finding is a laboratory artifact with the sTfR assay or if it represents real differences in iron homeostasis.

Iron supplements were reported to be taken by 39% of RISE donors. Since the actual multivitamin or mineral supplements were not audited to determine their iron content, this result needs to be interpreted with some caution. However, such donors had a significantly lower prevalence of AIS in the adjusted model suggesting that low dose iron supplements may be beneficial in ameliorating iron depletion. However, the actual magnitude of the effect was small (15.2% AIS in non-supplement donors vs. 14.9% AIS in supplemented donors).

HFE genotype was independently associated with IDE in the adjusted model. However, the effect appears to be due to homozygote or mixed heterozygote genotype for C282Y and H63D. A transferrin polymorphism, G277S, which has been associated with an increased prevalence of iron deficiency anemia in menstruating women,⁴ was not associated with AIS or IDE. A more detailed genetic analysis of the RISE donors is planned.

A variety of dietary variables were included in the model. The foods chosen for inquiry were primarily iron-rich foods. However, the only food that was significant in the multivariable model was other fish (defined as consumption of fish other than iron-rich clams, oysters, mussels, shrimp and sardines). Surprisingly beef and liver consumption, which are often recommended to blood donors, had little to no effect after adjusting for other factors. It would appear based on the self-reported RISE dietary variables, that consumption of these foods does not have a meaningful impact on blood donor iron status. More comprehensive dietary surveys would have been desirable, but RISE data was limited by the desire to restrict the length and complexity of the questionnaire. Therefore the lack of dietary effects may be due to the inaccuracy of our measures of iron-rich food consumption, especially given that donors were asked to generalize about their dietary habits over a long period of time. It is also possible that diet would be more important in other countries. Nevertheless, diet does not seem to be a particularly fruitful area for this investigation given the data from our dietary questionnaire, but analysis of the longitudinal data will be important to verify this.

The six REDS-II centers each enrolled an approximately equal number of donors. When blood center was included in the multivariable model, center E was associated with higher levels of AIS and IDE, even after adjustment for multiple donor characteristics that may differ between centers. Since center A was the only center using 450 mL whole blood collection containers, we also analyzed the results from center A versus the other 5 centers combined. However, no significant difference in the odds of AIS or IDE was found in center A donors compared to donors at the other 5 centers, and the remaining 5 centers continued to show difference among them in the odds of AIS and IDE, after center A was removed. The explanation for this overall finding is not certain. It is possible that unrecognized methodological differences existed between center E and the others or that the statistical model did not adequately control for underlying donor differences between centers. The finding of differences among centers illustrates the shortcomings of single center studies since a study done at center E might come to different conclusions than at the other centers. As the REDS-II centers are geographically diverse and chosen to represent the U.S. blood supply, RISE as a multi-center study is likely to better reflect the characteristics of U.S. blood donors as a whole than single center studies.

A limitation for the current analysis is the cross-sectional nature of the enrollment data. This raises the possibility that previously deferred donors may no longer be donating; hence, the magnitude of iron depletion and or anemia may be underestimated. Other possible limitations include errors inherent in self-reported weight and diet. Finally, the use of peripheral blood assays, rather than bone marrow examinations, to assess donor iron status leaves open the possibility of interpretation error based on inferences about donor iron status

(AIS and IDE). However, the RISE study is a large, geographically diverse evaluation of blood donor iron status which includes a comprehensive evaluation of donor demographic, genetic, and behavioral factors and their relationship to iron deficiency. As such it is a unique resource for understanding factors that impact on donor iron stores.

In summary, the enrollment data from RISE confirm previous studies documenting a high prevalence of AIS and IDE in frequent blood donors and confirm the strong association between prior donation intensity and the presence of AIS/IDE. In addition to the well known relationship between iron depletion and female gender as a result of menstruation and pregnancy, AIS and IDE in donors are also significantly associated with donor weight, presumably as an indicator of red cell mass. These three factors together are readily apparent in the individuals presenting at blood drives and could be readily manipulated to reduce the prevalence of AIS or IDE by altering standards for frequency of whole blood and double red cell collection to relate allowable donation frequency to donor gender and size. Iron supplementation of blood donors has been proposed for routine implementation and several pilot operational and clinical trials have been conducted.⁹ However, operational as well as medical/legal/ethical obstacles have prevented widespread implementation of iron supplementation to date. Based on this study, the impact of low dose iron supplementation could be relatively modest, in part because many blood donors appear to already been taking supplements; on the other hand, it is possible that an increased dose, or programs that ensure donor compliance may still be beneficial. A comprehensive multi-center clinical trial of iron supplementation is warranted. This study suggests that other potential interventions, such as changing the consumption of iron-rich foods or alteration of smoking habits, would likely not be effective. Finally selecting donors on the basis of inherent factors such as race/ethnicity, country of birth and genetic makeup (as far as can be measured) would appear to have limited influence on AIS/IDE.

Given the widespread and frequent occurrence of blood donation, optimizing its safety for volunteer donors requires significant attention. It appears likely that the findings reported here will stimulate analysis of the health importance of iron depletion in blood donors and the ability of the current donor standards to prevent and detect significant adverse donor sequelae related to iron depletion. They should also cause consideration of interventions to better manage donor iron balance. Reducing the frequency of blood donation would reduce the prevalence of iron deficiency among blood donors, as might implementing routine iron supplementation. In addition to the data presented here, decisions on these interventions will depend on the prospective data obtained after follow-up of the RISE cohort is completed as well as the assessment of the health significance of iron deficiency.

Acknowledgments

The authors thank the staff at all six participating blood centers. Without their help, this study would not have been possible.

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

References

1. Simon TL, Garry PJ, Hooper EM. Iron stores in blood donors. JAMA 1981;245:2038–2043. [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;48:2197. [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;50:441–447. [PubMed: 884321]

4. 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;115:329–333.
5. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and Iron-Overload Screening in a Racially Diverse Population. *N Engl J Med* 2005;352:1769–78. [PubMed: 15858186]
6. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genetics* 1996;13:399–408. [PubMed: 8696333]
7. Newman B. Iron depletion by whole-blood donation harms menstruating females: The current whole-blood-collection paradigm needs to be changed. *Transfusion* 2006;46:1667–1681. [PubMed: 17002622]
8. Sullivan JL. Stored iron and vascular reactivity. *Arterioscler Thromb Vasc Biol* 2005;25:1532–1535. [PubMed: 16055755]
9. Bianco C, Brittenham G, Gilcher RO, et al. Maintaining iron balance in women blood donors of childbearing age: summary of a workshop. *Transfusion* 2002;42:798–805. [PubMed: 12147035]
10. California Smoking Survey. 2002 [Accessed on 4/5/2010]. At http://webtecc.etr.org/cstats/base/publications/docs/Survey_CTS2002_Eng_Screener.pdf
11. NIH Diet History Questionnaire. 2002 [Accessed on 4/5/2010]. At <http://riskfactor.cancer.gov/DHQ/forms/files/shared/dhq1.2002.sample.pdf>
12. NHANES Reproductive Health questionnaire. 2000 [Accessed on 4/5/2010]. At http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/CAPI_RHQVB.pdf
13. Mansfield PK, Voda A, Allison G. Validating a pencil-and-paper measure of perimenopausal menstrual blood loss. *Women's Health issues* 2004;14:242–247.
14. Melmed R, Cable RG, Wright DJ. Modeling pre-donation venous hemoglobin from post-donation venous samples. (abstract). *Transfusion* 2009;49(3 supplement) Abstract S98, 41A.
15. Punnonen K, Irjala K, Rajamäki A. Serum transferrin receptor and its ratio to serum ferritin in diagnosis of iron-deficiency anemia. *Blood* 1997;89:1052–57. [PubMed: 9028338]
16. Punnonen K, Rajamäki A. Evaluation of iron status of Finnish blood donors using serum transferrin receptor. *Transfus Med* 1999;9:131–4. [PubMed: 10354382]
17. 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;45:5–10. [PubMed: 15647011]
18. Ali MA, Luxton AW, Walker WH. Serum ferritin concentration and bone marrow iron stores: a prospective study. *CMA Journal* 1978;118:945–946.
19. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA. Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood* 1990;75:102–107. [PubMed: 2294984]
20. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood* 1990;75:1870–1876. [PubMed: 2331526]
21. Mast A, Schlumpf K, Wright D, Custer B, Spencer B, Murphy E, Simon T. Demographic Correlates of Low Hematocrit Deferral among Prospective Whole Blood Donors. *Transfusion*. 2010 In Press.
22. American Association of Blood Banks. Standard 5.4.1A. 26. AABB; Bethesda MD: 2009. Standards for Blood Banks and Transfusion Services; p. 56
23. Code of Federal Regulations. 21 CFR 640.3 (b) (3).
24. Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? *Blood* 2006;107:1747–1750. [PubMed: 16189263]
25. Dallman PR, et al. Prevalence and causes of anemia in the United States, 1976 to 1980. *Am J Clin Nutr* 1984;39:437–445. [PubMed: 6695843]
26. Ali AM, Goldsmith CH, Mcavoy AT, Ali MAM, Blajchman MA. A prospective study evaluating the lowering of hemoglobin standards for blood donors. *Transfusion* 1989;29:268–272. [PubMed: 2922794]
27. 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]

28. Pynaert I, De Bacquer D, Matthys C, Delanghe J, Temmerman M, De Backer G, 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]

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
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
3. Other than today, when was the last time you donated blood?

Month Year
☐ Don't Know
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
5. Were any of these donations made through a DIFFERENT blood center?
☐ Yes
☐ No
☐ 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
were apheresis
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
8. Did you smoke ANY cigarettes during the last 90 DAYS (3 months)?
☐ Yes
☐ No (SKIP TO SECTION C QUESTION 11)
☐ 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
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, Theragen, 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?

- ☐ No
☐ Don't Know

☐ Yes

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

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
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
3. Other than today, when was the last time you donated blood?
- _____
 Month Year
- ☐ Don't Know
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
5. Were any of these donations made through a DIFFERENT blood center?
- ☐ Yes
☐ No
☐ 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
8. Did you smoke ANY cigarettes during the last 90 DAYS (3 months)?
- ☐ Yes
☐ No **(SKIP TO SECTION C QUESTION 11)**
☐ 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
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?

- ☐ No
☐ Don't Know

☐ Yes

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
☐ 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

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, Theragen, 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

- ☐ 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

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

Appendix B: Remaining odds ratios and p-values from the adjusted logistic regression model not presented in the main text

	AIS model	IDE model
	OR (95% CI)	OR (95% CI)
Race/Ethnicity		

	AIS model	IDE model
	OR (95% CI)	OR (95% CI)
<i>p-value</i>	0.96	0.52
White	1.0	1.0
Asian	0.9 (0.4–2.2)	0.8 (0.4–1.7)
Black	1.0 (0.5–2.0)	1.4 (0.8–2.3)
Hispanic	1.1 (0.5–2.3)	1.4 (0.8–2.6)
Other	1.7 (0.6–5.1)	1.4 (0.5–3.5)
Education		
<i>p-value</i>	0.12	0.49
≤ High School/GED	1.0	1.0
Some College/Associate Degree	1.7 (1.0–2.7)	1.3 (0.9–1.8)
College Degree or greater	1.4 (0.9–2.2)	1.4 (1.0–1.9)
Age ≤22	1.5 (0.6–3.7)	1.4 (0.7–2.8)
Country of Birth		
<i>p-value</i>	0.18	0.04
US Born	1.0	1.0
Not US Born	0.5 (0.2–1.0)	0.5 (0.3–0.9)
Aspirin		
<i>p-value</i>	0.35	0.58
Does not take daily	1.0	1.0
For heart health only	0.7 (0.5–1.1)	1.1 (0.8–1.4)
For pain or both	1.1 (0.6–2.0)	1.3 (0.8–2.2)
G277S Genotype		
<i>p-value</i>	0.97	0.22
Wild-Type	1.0	1.0
Heterozygous	1.0 (0.7–1.5)	1.1 (0.8–1.5)
Homozygous	****	0.3 (0.0–4.6)
In the past 12 months, how many times per week did you eat ...?		
Beef		
<i>p-value</i>	0.51	0.06
Never/Less than once a week	1.0	1.0
Once or twice a week	0.8 (0.6–1.1)	0.7 (0.5–0.9)
3 or more times a week	0.9 (0.6–1.3)	0.7 (0.5–1.0)
Dairy		
<i>p-value</i>	0.14	0.54
Never thru 4 times a week	1.0	1.0
5–6 times a week/Every day	0.8 (0.6–1.1)	0.9 (0.7–1.2)
Two or more times a day	1.1 (0.8–1.5)	1.1 (0.8–1.4)
Eggs		
<i>p-value</i>	0.24	0.72
Never/Less than once a week	1.0	1.0
Once or twice a week	1.4 (1.0–1.9)	1.1 (0.8–1.4)

	AIS model OR (95% CI)	IDE model OR (95% CI)
3 or more times a week	1.3 (0.8–1.9)	1.0 (0.7–1.3)
Lamb, Pork, Chicken or Turkey		
<i>p-value</i>	0.89	0.50
Never/Less than once a week/once a week	1.0	1.0
2–4 times a week	1.1 (0.7–1.4)	1.0 (0.8–1.3)
5 or more times a week	1.0 (0.7–1.7)	1.2 (0.9–1.7)
Liver		
<i>p-value</i>	0.78	0.32
Never Eats	1.0	1.0
Ever Eats	0.9 (0.7–1.3)	1.0 (0.8–1.3)
Clams		
<i>p-value</i>	0.20	0.45
Never	1.0	1.0
Less than once a week	0.9 (0.6–1.2)	1.0 (0.8–1.3)
Once a week or more	0.2 (0.0–1.0)	0.8 (0.4–1.6)
Oysters, Mussels, Shrimp or Sardines		
<i>p-value</i>	0.94	0.64
Never	1.0	1.0
Less than once a week	1.1 (0.8–1.5)	0.8 (0.6–1.1)
Once a week or more	1.0 (0.6–1.7)	0.8 (0.6–1.2)
Other Fish		
<i>p-value</i>	0.18	0.04
Never	1.0	1.0
Less than once a week	1.2 (0.7–2.0)	0.6 (0.4–0.8)
Once a week or more	1.5 (0.9–2.5)	0.6 (0.4–0.9)

Appendix

The Retrovirus Epidemiology Donor Study - II (REDS-II Study Group) is the responsibility of the following persons:

Blood Centers

American Red Cross Blood Services, New England Region

R. Cable, J. Rios and R. Benjamin

American Red Cross Blood Services, Southern Region/Department of Pathology and Laboratory Medicine, Emory University School of Medicine

J.D. Roback

Hoxworth Blood Center, University of Cincinnati Academic Health Center

R.A. Sacher, S.L. Wilkinson and P.M. Carey

Blood Centers of the Pacific, University of California San Francisco, Blood Systems Research Institute

E.L. Murphy, B. Custer and N. Hirschler

The Institute for Transfusion Medicine

D. Triulzi, R. Kakaiya and J. Kiss

Blood Center of Wisconsin

J.L. Gottschall and A.E. Mast

Coordinating Center: Westat, Inc

J. Schulman and M. King

National Heart, Lung, and Blood Institute, NIH

G.J. Nemo

Central Laboratory: Blood Systems Research Institute

M.P. Busch and P. Norris

Figure 1.
Effect of 12 month Whole Blood/Red Cell donation frequency on venous hemoglobin by males and pre/post menopausal females

Figure 2. Effect of 12 month Whole Blood/Red Cell donation frequency on the geometric mean of plasma ferritin by males and pre/post menopausal females

Medians (2.5%–97.5% Range) for Venous Hemoglobin, Ferritin, Soluble Transferrin Receptor (sTfR) and Log (sTfR/Ferritin) and proportion with Absent Iron Stores (AIS) and Iron Deficiency Erythropoiesis (IDE) by Enrollment Cohort

Table 1

Cohort	Hemoglobin g/dL*	Ferritin ng/mL	sTfR mg/L	Log (sTfR/Ferritin)	AIS	IDE
FT/RA Females	13.3 (11.5–15.2)	37 (9–175)	2.7 (1.7–4.8)	1.8 (1.1–2.6)	6.4%	24.7%
Frequent Females	13.2 (11.4–15.2)	19 (5–68)	3.1 (1.8–6.6)	2.2 (1.5–3.0)	27.1%	66.1%
FT/RA Males	15.1 (12.8–17.4)	108 (29–430)	2.7 (1.6–4.4)	1.4 (0.8–2.1)	0%	2.5%
Frequent Males	14.5 (12.0–16.6)	25 (6–117)	3.1 (1.8–8.0)	2.1 (1.3–3.1)	16.4%	48.7%

* Post donation venous hemoglobin samples were converted to pre-donation values using the formula $\text{Pre vHb(g/dL)} = \text{Post vHb} + 0.8423 - (0.002035 \times \text{Weight (lbs)})$ for the two centers where post donation hemoglobin and not pre donation hemoglobin was obtained.

Table 2

Absent Iron Stores (AIS) and Iron Deficiency Erythropoiesis (IDE) at Enrollment by Donor Characteristics

Panel A – Demographic 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*						
<i>p-value</i>			<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)						
<i>p-value</i>			0.04		<0.0001	
20		63	70	1 (1.6)	15 (21.4)	10 (15.9)
20–29		164	179	13 (7.9)	43 (24.2)	38 (23.2)
30–39		165	175	17 (10.3)	32 (18.3)	49 (29.7)
40–49		215	282	19 (8.8)	54 (19.1)	57 (26.5)
50–59		306	320	48 (15.7)	62 (19.4)	125 (40.9)
60+		262	224	28 (10.7)	33 (14.7)	105 (40.1)
Race/Ethnicity†						
<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)	

Panel A – Demographic Characteristics			
	Number of Donors	Number and Percentage with AIS	Number and Percentage with IDE
Other	28	5 (17.9)	11 (39.3)
Education[†]			
<i>p-value</i>		0.21	0.002
≤ High School/GED	292	34 (11.6)	110 (37.7)
Some College/Associate Degree	615	97 (15.8)	243 (39.5)
College Degree or greater	1130	184 (16.3)	515 (45.6)
Age ≤22	246	30 (12.2)	83 (33.7)
Country of Birth[†]			
<i>p-value</i>		0.12	0.006
US Born	2301	356 (15.5)	976 (42.4)
Not US Born	110	9 (8.2)	33 (30.0)
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)

Panel B: Dietary Intake			
	Number of Donors	Number and Percentage with AIS	Number and Percentage with IDE
In the past 12 months, how many times per week did you eat ...?			
Beef			
<i>p-value</i>		0.11	0.0002
Never/Less than once a week	463	86 (18.6)	232 (50.1)
Once or twice a week	1181	173 (14.7)	488 (41.3)
3 or more times a week	738	100 (13.6)	273 (37.0)
Dairy			
<i>p-value</i>		0.02	0.03

Panel B: Dietary Intake			Number of Donors	Number and Percentage with AIS	Number and Percentage with IDE
Never thru 4 times a week			695	101 (14.5)	287 (41.3)
5–6 times a week/Every day			1007	134 (13.3)	395 (39.2)
Two or more times a day			688	127 (18.5)	317 (46.1)
Eggs					
<i>p-value</i>				0.33	0.38
Never/Less than once a week			574	78 (13.6)	245 (42.7)
Once or twice a week			1273	207 (16.3)	543 (42.7)
3 or more times a week			508	72 (14.2)	195 (38.4)
Lamb, Pork, Chicken or Turkey					
<i>p-value</i>				0.86	0.73
Never/Less than once a week/once a week			456	69 (15.1)	190 (41.7)
2–4 times a week			1472	220 (15.0)	606 (41.2)
5 or more times a week			394	63 (16.0)	174 (44.2)
Liver					
<i>p-value</i>				0.25	0.03
Never Eats			1849	288 (15.6)	782 (42.3)
Ever Eats			509	71 (14.0)	212 (41.7)
Clams					
<i>p-value</i>				0.10	0.05
Never			1303	207 (15.9)	564 (43.3)
Less than once a week			933	133 (14.3)	365 (39.1)
Once a week or more			55	2 (3.6)	18 (32.7)
Oysters, Mussels, Shrimp or Sardines					
<i>p-value</i>				0.96	0.04
Never			612	94 (15.4)	285 (46.6)
Less than once a week			1403	212 (15.1)	559 (39.8)
Once a week or more			306	43 (14.1)	122 (39.9)
Other Fish					
<i>p-value</i>				0.70	0.002
Never			199	30 (15.1)	108 (54.3)
Less than once a week			934	131 (14.0)	372 (39.8)

Panel B: Dietary Intake			
	Number of Donors	Number and Percentage with AIS	Number and Percentage with IDE
Once a week or more	1249	198 (15.9)	515 (41.2)
Panel C: Weight, Smoking History, Medications, HFE and Transferrin Gene Mutation Status and for Females, Menstrual and Pregnancy History			
	Number of Donors	Number and Percentage with AIS	Number and Percentage with IDE
Weight*			
<i>p-value</i>			
<150	572	<0.0001	<0.0001
150–174	595	125 (21.9)	291 (50.9)
175–199	510	89 (15.0)	253 (42.5)
200+	627	74 (14.5)	210 (41.2)
Smoking*			
<i>p-value</i>			
Current	297	0.11	<0.0001
Past	613	38 (12.8)	93 (31.3)
Never	1413	79 (12.9)	234 (38.2)
Aspirin*			
<i>p-value</i>			
Does not take daily	1922	234 (16.6)	645 (45.7)
For heart health only	360	0.09	0.60
For pain or both	106	306 (15.9)	800 (41.6)
Iron Supplementation			
<i>p-value</i>			
Takes supplemental iron (multivitamin or Fe supp.)	954	39 (10.8)	151 (41.9)
No supplemental iron	1471	16 (15.1)	48 (45.3)
G277S Genotype*			
<i>p-value</i>			
Wild-Type	2107	0.85	0.03
Heterozygous	249	142 (14.9)	424 (44.4)
Homozygous	5	223 (15.2)	587 (39.9)
HFE (C282Y, H63D)*			
<i>p-value</i>			
		0.99	0.55
		318 (15.1)	876 (41.6)
		37 (14.9)	103 (41.4)
		0 (0)	1 (20.0)
		0.81	0.17

Panel C: Weight, Smoking History, Medications, HFE and Transferrin Gene Mutation Status and for Females, Menstrual and Pregnancy History			
	Number of Donors	Number and Percentage with AIS	Number and Percentage with IDE
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)
Homozygous for one or Heterozygous for both	87	11 (12.6)	26 (29.9)
Menstrual Status (females only) *			
<i>p-value</i>		0.12	0.13
Periods Stopped	566	98 (17.3)	272 (48.1)
Still has Period, but not at enrollment	597	120 (20.1)	310 (51.9)
Menstruating at enrollment	68	19 (27.9)	39 (57.4)
Pregnancy Status (females only) *			
<i>p-value</i>		0.96	0.49
Never Pregnant	405	80 (19.8)	192 (47.4)
Last pregnancy more than 5 years ago	666	127 (19.1)	350 (52.6)
Pregnancy in the last five years	90	15 (16.7)	44 (48.9)
Pregnant at some point, no date of pregnancy given	76	14 (18.4)	35 (46.0)

* Red cell donations is the number of whole blood and double red cell (counted as 2) donations that donor made in the 2 years prior to enrollment.

[†] Missing totals: Race/Ethnicity=18, Education=142, Country of Birth=14

* missing values: Beef=43, Dairy=35, Eggs=70, LPCT=103, Clams=134, Liver=67, Oysters, Mussels, Shrimp or Sardines=104, Other Fish=43

* Missing values: Weight=121, Smoking=102, Aspirin=37, G277S=64, HFE=3, Menstrual=19, Pregnancy=13

Table 3

Adjusted Odds Ratios (ORs) and 95% Confidence Intervals for AIS and IDE – Variables of Interest

	AIS model		IDE model	
	OR (95% CI)		OR (95% CI)	
Red Cell Donations in 2 years prior to enrollment				
<i>p-value</i>	<0.0001		<0.0001	
FT: 0 donations	1.0		1.0	
RA: 0 donations	0.5 (0.2–1.2)		1.5 (0.9–2.4)	
≤ 4 donations	5.3 (2.8–10.1)		14.0 (8.6–22.7)	
5–6 donations	12.5 (6.4–24.6)		24.0 (14.3–40.5)	
7–9 donations	13.5 (6.8–26.6)		32.3 (19.2–54.5)	
10+ donations	18.9 (9.0–39.6)		50.5 (28.4–89.9)	
Gender				
<i>p-value</i>	<0.003		<0.0001	
Male	1.0		1.0	
Female	1.8 (1.2–2.6)		2.8 (2.1–3.7)	
Age (in years)				
	Male	Female	Male	Female
<i>p-value</i>	0.31	<0.0001	0.01	<0.0001
<20	0.4 (0.0–3.3)	3.1 (1.0–9.6)	1.1 (0.7–5.9)	4.9 (1.9–12.5)
20–29	1.8 (0.8–4.2)	3.9 (2.0–7.6)	2.3 (1.2–4.3)	3.1 (1.8–5.4)
30–39	1.5 (0.7–3.2)	1.6 (0.9–3.0)	1.7 (1.0–2.9)	1.7 (1.0–2.8)
40–49	1.0	1.0	1.0	1.0
50–59	1.6 (0.9–2.9)	1.0 (0.6–1.7)	1.4 (0.9–2.2)	0.8 (0.5–1.2)
60+	0.9 (0.5–1.9)	0.7 (0.3–1.3)	1.2 (0.8–2.0)	0.8 (0.5–1.4)
Center				
<i>p-value</i>	0.01		<0.0001	
A	1.0		1.0	
B	1.1 (0.7–1.9)		1.1 (0.8–1.7)	
C	0.8 (0.5–1.2)		1.0 (0.7–1.4)	
D	1.1 (0.7–1.7)		1.1 (0.8–1.5)	
E	1.9 (1.2–3.0)		2.2 (1.5–3.1)	
F	1.1 (0.7–1.7)		1.4 (1.0–2.0)	
Weight				
<i>p-value</i>	0.0001		0.04	
<150	1.3 (0.9–1.9)		1.1 (0.8–1.5)	
150–174	1.0		1.0	
175–199	0.9 (0.6–1.3)		0.9 (0.7–1.2)	
200+	0.5 (0.3–0.8)		0.7 (0.5–0.9)	
Smoking				
<i>p-value</i>	0.59		0.0001	
Never	1.0		1.0	
Past	0.8 (0.6–1.1)		0.7 (0.5–0.9)	

	AIS model	IDE model
	OR (95% CI)	OR (95% CI)
Current	0.9 (0.6–1.3)	0.5 (0.4–0.8)
Iron Supplementation		
<i>p-value</i>	0.01	0.11
No Iron	1.0	1.0
Takes supplemental iron (multivitamin or Fe supp.)	0.7 (0.5–0.9)	0.8 (0.7–1.0)
HFE (C282Y, H63D)		
<i>p-value</i>	0.92	0.02
Wild-Type	1.0	1.0
Heterozygous – C282Y	1.0 (0.6–1.5)	0.9 (0.6–1.3)
Heterozygous – H63D	1.0 (0.7–1.4)	0.9 (0.7–1.1)
Homozygous for one or Heterozygous for both	0.7 (0.3–1.4)	0.4 (0.2–0.7)
Menstrual Status		
<i>p-value</i>	0.14	0.05
Periods Stopped	1.0	1.0
Still has Period, but not at enrollment	1.1 (0.7–1.8)	1.6 (1.0–2.4)
Menstruating at enrollment	2.0 (0.9–4.4)	2.0 (1.0–4.1)
Pregnancy Status		
<i>p-value</i>	0.09	0.0004
Never Pregnant	1.0	1.0
Last pregnancy more than 5 years ago	1.4 (0.9–2.2)	2.0 (1.4–3.0)
Pregnancy in the last five years	1.5 (0.7–3.1)	2.2 (1.2–4.1)
Pregnant at some point, no date of pregnancy	1.2 (0.6–2.6)	1.5 (0.8–2.8)

[†]The gender effect compares male donors averaged over the observed male age distribution to female donors averaged over the observed female age, menstrual, and pregnancy distributions.