

Behavioral, biochemical, and genetic analysis of iron metabolism in high-intensity blood donors

Alan E. Mast, Tisha M. Foster, Holly L. Pinder, Craig A. Beczkiewicz, Daniel B. Bellissimo, Anthony T. Murphy, Steve Kovacevic, Victor J. Wroblewski, and Derrick R. Witcher

BACKGROUND: Individuals donating whole blood 13 times in a 2-year period without development of iron deficiency anemia (superdonors) are a self-selected population that is deferred for low hematocrit (Hct) level less frequently than other donors.

STUDY DESIGN AND METHODS: Iron metabolism was assessed in 138 superdonors through a questionnaire and measurement of Hct, serum ferritin, serum hepcidin, and serum growth differentiation factor 15 (GDF15). Genetic testing for HFE and JAK-2 mutations was also performed.

RESULTS AND CONCLUSIONS: Iron deficiency (ferritin level, $<30 \mu\text{g/L}$) is present in more than 60 percent of superdonors. Behaviors altering iron status included casual use of iron supplements in males, but not in females, and cigarette smoking that produced increased Hct associated with decreased ferritin. The striking biochemical characteristic of superdonors is greatly decreased serum hepcidin, consistent with their need to absorb maximal amounts of dietary iron to replace that lost from blood donation. GDF15 is normal in most superdonors, indicating that GDF15 overexpression arising from the expanded erythroid pool necessary to replace donated red cells is not the biochemical mechanism for the decreased serum hepcidin. Mutations in JAK-2 were not found, indicating that undiagnosed polycythemia vera is not a common cause for successful repeated blood donation by superdonors. Mutations in *HFE* associated with hemochromatosis were present in superdonors at the same frequency as the normal population. However, superdonors heterozygous for the H63D mutation in *HFE* had significantly decreased hepcidin : ferritin ratios demonstrating for the first time that the heterozygous state for *HFE* mutations is associated with alterations in hepcidin expression.

Whole-blood donors are allowed to donate blood every 56 days losing 200 to 250 mg of iron with each donation.¹ Individuals repeatedly donating every 56 days over an extended period of time must absorb an additional 3.5 to 4.5 mg of iron per day to replace that lost through blood donation and avoid development of iron deficiency. This represents a four- to fivefold increase in the daily iron needs for males and postmenopausal females and a two- to threefold increase for menstruating females. This amount meets or exceeds the reported maximal iron absorption rates, regardless of iron intake, of 3.99 mg per day for men and 3.79 mg per day for women.² It also is more daily iron absorption than occurs in patients with hereditary hemochromatosis, a disease associated with hyperabsorption of iron irrespective of need, who typically absorb an additional 1 to 3 mg of iron per day. Nevertheless, many healthy individuals are able to repeatedly donate whole blood every 56 days without deferral for low hematocrit (Hct) level despite the increased iron requirement for production of new red blood cells (RBCs).

Frequent donors are critical to maintaining the blood supply and often are called upon to donate in the face of periodic blood shortages. These donors are at risk for low Hct deferral, the most common reason that a potential blood donor is not allowed to donate. If new strategies to

ABBREVIATION: GDF15 = growth differentiation factor 15.

From the Blood Research Institute, Blood Center of Wisconsin, and the Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin; BloodNet USA, Lakeland, Florida; and the Biotechnology Discovery Research, Lilly Research Laboratories, Indianapolis, Indiana.

Address reprint requests to: Alan E. Mast, Blood Research Institute, 8727 Watertown Plank Road, Milwaukee, WI 53226-3548; e-mail: alan.mast@bcw.edu.

Received for publication March 5, 2008; revision received April 22, 2008; and accepted April 22, 2008.

doi: 10.1111/j.1537-2995.2008.01823.x

TRANSFUSION 2008;48:2197-2204.

prevent iron deficiency anemia in blood donors can be developed, it would lead to healthier blood donors and a more robust blood supply. We studied iron metabolism in highly successful whole-blood donors to define key characteristics contributing to their success that can be applied to the broader population of blood donors. Information on iron ingestion, menstrual status, and smoking status was obtained from 138 individuals who successfully donated whole blood 13 times within a 2-year period (superdonors). Blood samples were analyzed for Hct, serum ferritin, serum hepcidin, and serum growth differentiation factor 15 (GDF15) as well as mutations in *HFE* that are associated with development of hemochromatosis³ and the *JAK2* V617F mutation that is associated with polycythemia.⁴ The data obtained from this study provide the basis for understanding the behavioral, biochemical, and genetic characteristics of individuals able to repeatedly donate whole blood without developing iron deficiency anemia.

MATERIALS AND METHODS

Effect of blood donation on low Hct deferral

Individuals donating whole blood at Blood Center of Wisconsin were tracked in an electronic database between January 1, 2004, and December 31, 2005 (>150,000 individuals). They were separated into groups based on the number of successful whole-blood donations (1 to 13) during this period. Platelet donations were not included in the analysis. Donors were then stratified by sex. Females were further stratified by age assuming that women more than 50 years old are postmenopausal. The proportion of donors in each group with low Hct deferral was determined by dividing the number of donors with one or more low Hct deferrals by the total number of donors in each donation intensity and demographic stratum.

Donor recruitment and sample collection

The Institutional Review Board of Blood Center of Wisconsin approved the studies. Individuals successfully donating whole blood 13 times between January 1, 2004, and December 31, 2005, were sent a letter asking them to participate in this study. Those responding and providing informed consent were enrolled and asked to complete a questionnaire providing information on diet, dietary supplements, smoking history, and family history of hemochromatosis. Female donors provided pregnancy history and menstrual status. Once enrolled, donors were tracked electronically and study investigators were notified the morning after donation. At this time, the result from the finger stick Hct was recorded, and a sample was obtained from remaining blood after completion of infectious disease testing.

Peripheral blood tests

Finger stick Hct was obtained at the time of donation via capillary spin. Serum ferritin analysis was performed on an immunology analyzer (Elecys, Roche, Indianapolis, IN) and calibrated to World Health Organization Standard 80/602. Serum hepcidin concentration was determined using liquid chromatography tandem mass spectrometry as previously described.⁵ A commercially available GDF15 enzyme-linked immunosorbent assay (ELISA) was used for quantification of GDF15 in serum samples (R&D Systems, Minneapolis, MN). The mean serum concentration for this assay in healthy volunteers is 450 ± 50 pg per mL.⁶

Genetic tests

The *JAK2* (Janus kinase 2) and *HFE* (hemochromatosis) molecular testing was performed by the Molecular Diagnostics Laboratory at Blood Center of Wisconsin using real-time polymerase chain reaction (LightCycler, Roche). The presence of the V617F mutation in Exon 12 of the *JAK2* gene and the H63D, S65C, and C282Y mutations in the *HFE* gene were determined by polymerase chain reaction amplification and hybridization with specific fluorescence resonance energy transfer probes. Melting curve analysis was then used to detect these sequence variations.

Statistical analysis of data

A double-tailed t test was used for assigning significance between groups. p Values of less than 0.05 were considered significant.

RESULTS

Low Hct deferral rates level off and begin to decrease with increased frequency of whole-blood donation

Analysis of the proportion of donors with low Hct deferral based on blood donation intensity and stratified by sex/ menstrual status is presented in Fig. 1. A notable feature of these plots is that the low Hct deferral rates level off and then begin to decrease with increased donation frequency. The effect is more dramatic for women where the deferral rates level off after only 4 to 5 donations compared to men where the rates increase in an almost linear manner through 12 donations. The difference between women and men is likely due to lower baseline iron stores in women produced by menstruation. These data suggest that frequent blood donors are a self-selected population with behavioral, biochemical, and/or genetic characteristics that allow for greater dietary iron absorption, and consequent less frequent deferral for low Hct, than the general population. A study was initiated to better understand iron metabolism in high-intensity blood donors.

Recruitment and demographic characteristics of study subjects

Blood donors who had successfully donated whole blood 13 times within a 2-year period were contacted. A total of 143 of 238 donors contacted responded and provided informed consent to participate in the study. A complete data set obtained on 138 (101 male and 37 female) of these subjects is presented here. All of the enrolled subjects are Caucasian except 2. The mean age of the male donors was 58.4 (range, 39 to 79) years and of female donors was 60.1

(range, 42 to 81) years. Of the females, 34 described themselves as postmenopausal, 3 as perimenopausal, and 4 as premenopausal.

Superdonors have depleted iron stores

The male and female distribution of Hct and ferritin values are presented in Fig. 2. Despite maintaining a Hct level of 38 percent or greater required for whole-blood donation, many of the donors have depleted iron stores. In otherwise healthy individuals, a serum ferritin level of less than 30 ng per mL has been identified as an indicator of absent iron stores.⁷ Using this definition, 61 of 101 (60.4%) of male and 24 of 37 (64.9%) of female superdonors were iron-deficient.

Use of iron supplements to donate blood

Oral iron supplement use was reported by 13 of 101 (12.9%) male and 16 of 37 (43.2%) female superdonors. In men this resulted in a significant increase in ferritin ($p = 0.012$) when compared to men not using iron supplements (Table 1). A similar increase in ferritin was not observed in women taking iron supplements (Table 2). Multivitamin with iron, but not oral iron supplement, use was reported by 26 of 101 (25.7%) male and 14 of 37 (37.8%) female superdonors. The use of multivitamins with iron did not produce a significant increase in ferritin values in either men or women (Tables 1 and 2). Ferritin was not elevated in superdonors reporting to eat red meat more than three times per week compared to those who did not (data not shown).

Smoking produces increased Hct with decreased iron stores

Thirteen of the study subjects, 11 males and 2 females, reported cigarette smoking within the previous 30 days. Hct and ferritin were analyzed in the 11 male smokers. When compared to male nonsmokers, smokers had significantly higher Hct ($p = 0.015$) and significantly lower ferritin ($p = 0.022$) than nonsmokers (Table 1).

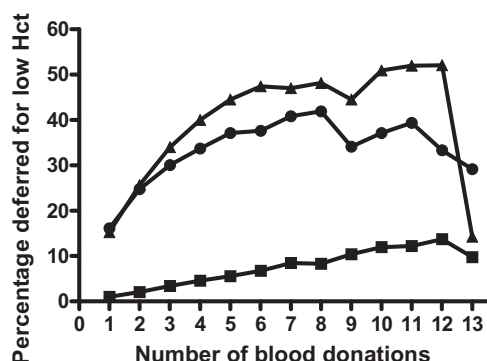


Fig. 1. Superdonors are a self-selected population with lower than expected deferral for low Hct. The percentage of men (■), women under age 50 (▲), and women over age 50 (●) deferred at least one time for low Hct stratified by donation intensity (1 to 13 whole-blood donations) over a 2-year period.

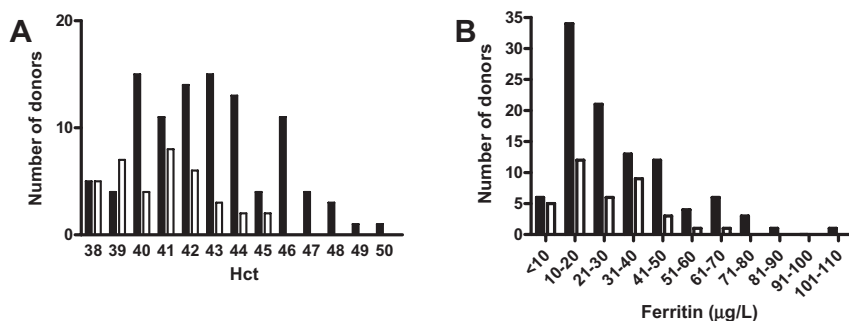


Fig. 2. Superdonors are iron-deficient despite Hct values acceptable for blood donation. (A) The number of donors with each Hct value is shown. (B) The number of donors in each range of serum ferritin is shown. (■) Males; (□) females.

TABLE 1. Male Hct, ferritin, and hepcidin values based on iron use and smoking (mean \pm SD)

Measure	All (n = 101)	No iron (n = 58)	MV plus iron (n = 26)	Iron supplementation (n = 13)	Smoker (n = 11)	Nonsmoker (n = 90)
Hct (%)	42.8 \pm 2.7	42.8 \pm 2.6	43.2 \pm 3.2	42.2 \pm 2.3	45.3 \pm 3.4†	42.5 \pm 2.4
Ferritin (ng/mL)	31.5 \pm 20	28.1 \pm 20	31.3 \pm 17	46.7 \pm 21*	24.8 \pm 7‡	32.4 \pm 21
Hepcidin (ng/mL)	2.41 \pm 3.8	2.28 \pm 3.9	2.09 \pm 2.7	4.02 \pm 5.3	1.32 \pm 2.7	2.55 \pm 3.9

* Significant ($p = 0.012$) compared to males not using iron supplements.

† Significant ($p = 0.015$) compared to nonsmokers.

‡ Significant ($p = 0.022$) compared to nonsmokers.

MV = multivitamin.

Serum hepcidin is greatly reduced in superdonors

Hepcidin is an iron regulatory hormone produced by the liver. It negatively regulates iron absorption from the gastrointestinal tract.⁸ Superdonors have greatly reduced serum hepcidin concentrations (Fig. 3A). Only 7 of 138 (5.1%) had serum hepcidin levels at or above the levels of hepcidin present in healthy volunteers (8 ng/mL),⁵ whereas in 55 of 138 (39.9%), serum hepcidin was not detectable (<1 ng/mL). A significant decrease in hepcidin was present in women not taking iron supplements when compared to those taking them ($p = 0.0012$; Table 2); however, a similar effect was not observed in male donors (Table 1). Serum hepcidin was not significantly different in superdonors reporting to eat red meat more than three times per week compared to those who did not (data not

shown). Comparison of ferritin and hepcidin for individual donors demonstrates some correlation between these measures of iron metabolism (Fig. 3B). Of the 67 donors with ferritin levels below 25 ng per mL, only 3 had hepcidin levels of more than 3.5 ng per mL and 41 had hepcidin levels of less than 1 ng per mL. As ferritin level increases to higher than 25 ng per mL, the number of donors with higher hepcidin also increases; however, numerous donors have hepcidin levels of less than 3.5 ng per mL despite having ferritin levels of higher than 25 ng per mL.

GDF15 levels do not correlate with ferritin or hepcidin

GDF15 is a member of the transforming growth factor (TGF)- β superfamily. GDF15 serum levels are increased in

conditions associated with an expanded erythroid compartment such as occurs in patients with β -thalassemia.⁶ GDF15 produced by maturing erythroblasts down regulates hepcidin production by the liver causing increased iron absorption from the gastrointestinal tract.⁶ Because superdonors have very low serum hepcidin levels and have an expanded erythroid compartment necessary to replace the RBCs lost through blood donation, we considered the possibility that elevated GDF15 may mediate the decrease in serum hepcidin. GDF15 levels varied widely in the superdonors. In male donors, the GDF15 serum concentration was 1261 ± 3162 pg per mL (range, 31 to 29,642 pg/mL); in female donors, it was 754 ± 945 pg per mL (range, 81 to 5168 pg/mL; Fig. 4). Although these means are higher than the reported mean serum concentration for healthy volunteers of 450 ± 50 pg per mL, they

TABLE 2. Female Hct, ferritin, and hepcidin values based on iron use (mean \pm SD)				
Measure	All (n = 37)	No iron (n = 7)	MV plus iron (n = 14)	Iron supplementation (n = 16)
Hct (%)	40.8 \pm 2.0	40.3 \pm 1.3	41.8 \pm 2.3	40.2 \pm 1.8
Ferritin (ng/mL)	25.2 \pm 15	21.6 \pm 21	28.3 \pm 17	24.1 \pm 10
Hepcidin (ng/mL)	2.76 \pm 2.8	0.486 \pm 0.83*	2.69 \pm 2.0	3.81 \pm 3.3

* Significant ($p = 0.0012$) compared to women using MV plus iron or iron supplements. MV = multivitamin.

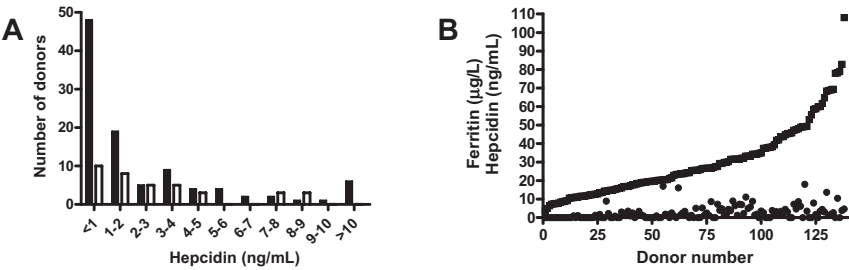


Fig. 3. Serum hepcidin is greatly reduced in superdonors. (A) The number of donors in each range of serum hepcidin is shown. (■) Males; (□) females. The mean serum hepcidin level in normal individuals is 8 ng per mL.⁵ (B) Comparison of serum hepcidin (●) with corresponding serum ferritin (■) in each superdonor is shown.

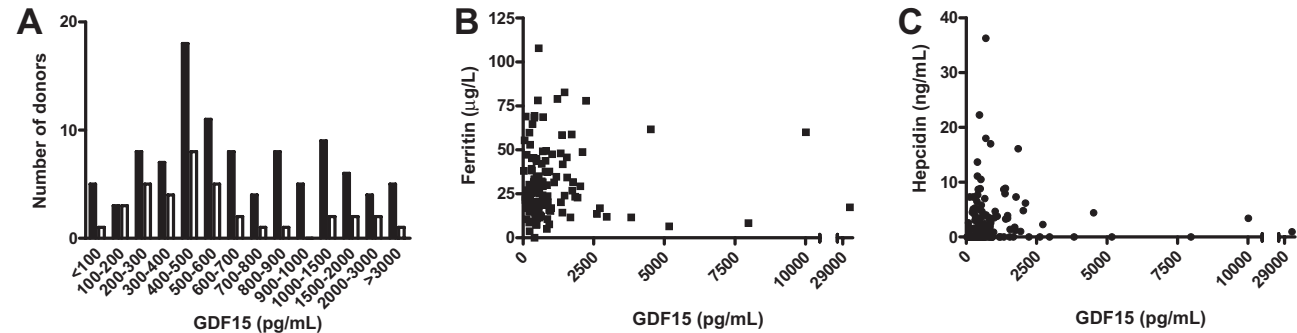


Fig. 4. Serum GDF15 is not consistently elevated in superdonors. (A) The number of donors in each range of serum GDF15 is shown. (■) Males; (□) females. (B) A plot of serum GDF15 versus serum ferritin demonstrating that the values do not correlate. (C) A plot of serum GDF15 versus serum hepcidin demonstrating that the values do not correlate.

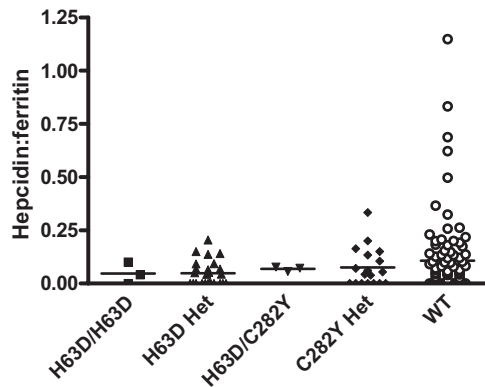


Fig. 5. Superdonors heterozygous for the H63D mutation in *HFE* have significantly reduced hepcidin : ferritin ratio. Hepcidin : ferritin ratio in superdonors with different *HFE* genotypes. *p* Values for each genotype when compared to wild type (WT) are H63D/H63D, 0.16; H63D heterozygous, 0.011; H63D/C282Y, 0.060; and C282Y heterozygous, 0.25.

are skewed by a small number of superdonors who had very high levels. Seventy (50.7%) of the superdonors had “normal” GDF15 serum concentrations (<550 pg/mL). There is no correlation between GDF15 and either hepcidin or ferritin (Fig. 4). The mean GDF15 concentration was not significantly different between donors using iron supplements and those who did not (data not shown).

The *HFE* H63D mutation is associated with decreased hepcidin : ferritin ratio

The C282Y mutation, and to a lesser degree the H63D mutation, in the *HFE* gene are associated with unregulated iron absorption and the development of hemochromatosis. The C282Y mutation is present in 21 (15.2%) of the superdonors, slightly more than the reported 10 to 12 percent prevalence for the general Caucasian population.⁹⁻¹¹ This increase did not reach significance. None of the superdonors were homozygous for C282Y. The hepcidin : ferritin ratio was decreased in donors heterozygous for C282Y but it did not reach significance (Fig. 5). The H63D mutation is present in 31 (20.3%) superdonors, slightly less than the reported 24 percent prevalence for the general population.⁹⁻¹¹ Three of the 31 are homozygous for H63D and 3 are double heterozygous for the two mutations. Superdonors heterozygous for the H63D mutation had a significant reduction in hepcidin : ferritin ratio when compared to those without the mutation (*p* = 0.011; Fig. 5). The *HFE* S65C mutation was present in 5 superdonors (3 males and 2 females). This was not significantly different from the general Caucasian population. Because of the low number of superdonors with this mutation, they were not analyzed further.

The *JAK-2* mutation was not identified in superdonors

The *JAK-2* V617F mutation is present in patients with polycythemia vera.⁴ This mutation was not found in any of the 138 superdonors, indicating that undiagnosed polycythemia vera is not a common cause for successful repeated blood donation by superdonors.

DISCUSSION

Approximately 14 million units of whole blood are collected annually by blood centers throughout the United States. As a result, approximately 3.5 tons of iron is removed from whole-blood donors each year. This loss of iron is evident in the decrease in the iron stores as measured by serum ferritin that is associated with whole blood donation.¹² Garry and coworkers² demonstrated that serum ferritin concentration decreases steadily through five blood donations and then stabilizes at a mean concentration of approximately 20 ng per mL. During the course of these donations, it appears that daily iron absorption gradually increases to the point that the donor's iron needs are met without further depletion of stores. However, there is individual variation in the ability to absorb dietary iron, and many frequent donors are deferred for low Hct. Superdonors are a self-selected population able to repeatedly donate whole blood without being deferred for low Hct despite having depleted iron stores, indicating that they have higher than expected absorption of dietary iron.

Simon and coworkers¹³ demonstrated that even casual use of iron supplements, such as that found in multivitamins, decreases the impact of blood donation on iron stores in menstruating women. These investigators went on to demonstrate that regular dietary supplementation with 39 mg per day elemental iron allowed menstruating women to donate blood as often as every 56 days.¹⁴ Studies such as these have led to calls for blood centers to provide a 56-day course of iron supplements to all menstruating women after successful donation.^{15,16} However, the finding of a beneficial effect of casual iron supplementation on iron stores has not been universal. In a study of individuals donating a mean of 15 units of blood over 3.5 years, a beneficial effect of casual iron supplementation was found in female, but not male, blood donors.² In this study, we also found variable effects of casual iron supplementation. Casual iron supplementation increased ferritin in males while in females it did not. Of 37 female superdonors, 30 reported taking either multivitamins with iron, iron supplements, or both whereas only 7 did not take any form of iron supplement. One of those not taking any supplemental iron was premenopausal and was severely iron-deficient with a ferritin level of 3.7 ng per mL. However, when compared as a group, the women taking iron did not have significantly increased

iron stores when compared to those not taking iron. Interestingly, the serum hepcidin level was significantly reduced in the women not taking iron. As discussed below, mutations in the *HFE* gene correlate with decreased serum hepcidin : ferritin ratio in superdonors. Only 2 of the 7 women not taking iron had a *HFE* mutation (one homozygous for H63D and one heterozygous for H63D), demonstrating that female superdonors are able to dramatically decrease their serum hepcidin level, allowing for maximal absorption of dietary iron regardless of their *HFE* mutation status.

Of the 101 male superdonors, 39 reported use of multivitamins with iron, iron supplements, or both whereas 62 did not take any form of iron. In contrast to the female superdonors, the use of iron supplements, but not the use of multivitamins with iron, significantly increased iron stores in male superdonors whereas hepcidin values were equivalent for all groups. It appears that a subgroup of the male superdonors have self-determined that use of iron supplements effectively allows them to repeatedly donate whole blood. Many male superdonors do not take any form of additional iron and are iron-deficient. This suggests that information about the impact of repeated blood donation on iron stores and benefits of the use of iron supplements is not widely appreciated by donors at Blood Center of Wisconsin and probably at most other blood centers across the United States.

Eleven of the male superdonors reported that they smoked cigarettes. These donors had significantly increased Hct values that were associated with significantly decreased iron stores. This is consistent with cigarette smoking causing hypoxia-induced erythrocytosis that decreases their likelihood for low Hct deferral despite the presence of iron deficiency. Cafolla and coworkers¹⁷ reported a similar finding in studies of folate in blood donors. Both serum folate and RBC folate were significantly lower in blood donors than nondonors and were further reduced in blood donors who smoked cigarettes.

Superdonors provide an opportunity to investigate iron metabolism in healthy people exposed to regular, repeated phlebotomy over an extended period of time. Hepcidin is a 25-amino-acid peptide produced by the liver that binds to ferroportin causing it to be internalized and degraded.¹⁸ Without ferroportin, enterocytes cannot release iron absorbed from the gastrointestinal tract into the blood stream. A striking biochemical characteristic of superdonors is their greatly reduced serum hepcidin concentration. The hepcidin values somewhat correlated with ferritin in that those with ferritin levels of less than 25 ng per mL all had hepcidin of less than 2 ng per mL whereas those with higher ferritin levels tended to have the detectable (nonzero) hepcidin values. The decreased serum hepcidin levels in superdonors is consistent with their need to absorb maximal amounts of dietary iron to replace that lost from blood donation.

Intestinal iron absorption is thought to be controlled by both an iron stores regulator and an erythroid regulator.¹⁹ The stores regulator has capacity to change iron balance by less than 2 mg per day whereas the capacity of the erythroid regulator is greater. The erythroid regulator will produce increased iron absorption, regardless of body iron stores, to defend against iron-deficient erythropoiesis.¹⁹ The erythroid regulator exerts its activity, at least in part, through suppression of hepcidin and is produced during periods of increased erythropoiesis.²⁰ One molecule that acts as an erythroid regulator is GDF15, a member of the TGF- β superfamily. GDF15 is released from the erythroid compartment and decreases hepatic hepcidin expression. Patients with β -thalassemia have an expanded erythroid compartment that produces greatly elevated serum GDF15 concentrations (66,000 pg/mL).⁶ This results in continual dietary iron absorption despite total body iron overload. We hypothesized that the decreased serum hepcidin concentration in superdonors partially results from increased serum GDF15 produced secondary to the expanded erythroid compartment necessary to replace RBCs lost through blood donation. This hypothesis proved to be incorrect. Most superdonors have GDF15 within the reported normal range and even those with the highest levels were well below that observed in patients with β -thalassemia. Because the life span of RBCs is 120 days and each whole-blood donation removes approximately 10 percent of the donor's blood, superdonors produce only 1.2-fold more RBCs than non-blood donors. This is a minimal erythropoietic stress compared to patients with β -thalassemia whose erythroid compartment may be expanded 10-fold. The superdonors also do not have ineffective erythropoiesis as occurs in patients with β -thalassemia and is thought to be a key component of greatly increased expression of GDF15.⁶ Thus, it is likely that a second mediator or combination of mediators down regulates hepcidin expression in superdonors.

Decreased hepcidin is found in all types of hemochromatosis (except ferroportin deficiency).⁸ Piperno and coworkers²¹ measured the urine hepcidin : ferritin ratio in 88 patients with hemochromatosis (61 C282Y/C282Y, 27 C282Y/H63D). Nonphlebotomized individuals either homozygous for the C282Y mutation or double heterozygous for the two mutations have decreased hepcidin: ferritin ratio compared to those without these mutations, demonstrating that these mutations alter iron sensing and subsequent hepcidin production. The C282Y and the H63D mutations in the heterozygous state are not present above the expected frequency in superdonors indicating that selection for these mutations is not responsible for their ability to repeatedly donate whole blood without low Hct deferral. However, those heterozygous for the H63D mutation have significantly decreased hepcidin: ferritin ratio when compared to superdonors without *HFE* mutations indicating that they are absorbing more iron at a

given ferritin level than would be expected for an individual without the mutation. Those with other *HFE* mutations had a trend toward a lower ratio that likely would become significant if more donors with these mutations were enrolled in the study. These data provide the first evidence that the heterozygous state for *HFE* mutations can produce alterations in hepcidin expression. Further studies will be required to determine if the decreased hepcidin : ferritin ratio in these superdonors is due to a decrease in baseline or an increased response to repeated phlebotomy.

Our findings demonstrate multiple reasons for the ability of some individuals to repeatedly donate whole blood without development of iron deficiency anemia. The use of iron supplements to ensure that adequate iron is available to replace that lost from blood donation is important for most female as well as many male donors. The decreased supplemental iron needs of males may be due, in part, to the 38 percent Hct donation criterion that allows men with anemia to donate blood. Cigarette smoking is a second behavioral characteristic that produces an increase in Hct, allowing frequent donation without deferral. This occurs at the expense of more severe iron deficiency than is observed in nonsmoking donors. In addition, there are genetic characteristics that alter the ability of individuals to decrease serum hepcidin to absorb maximal amounts of dietary iron. Some of these, such as the *HFE* mutations, are relatively well defined, whereas there undoubtedly are others that are yet to be identified. Superdonors will remain a unique and highly informative population of healthy individuals for study of these new regulators of iron metabolism as they are identified.

ACKNOWLEDGMENTS

The authors thank Marlene Kurek for administrative assistance and database management and Maureen Collins for performance of GDF15 ELISAs. The authors have no conflicts of interest.

REFERENCES

1. Simon TL. Iron, iron everywhere but not enough to donate. *Transfusion* 2002;42:664.
2. Garry PJ, Koehler KM, Simon TL. Iron stores and iron absorption: effects of repeated blood donations. *Am J Clin Nutr* 1995;62:611-20.
3. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolff RK. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399-408.
4. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, Garcon L, Raslova H, Berger R, Naceur-Griscelli A, Villeval JL, Constantinescu SN, Casadevall N, Vainchenker W. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005;434:1144-8.
5. Murphy AT, Witcher DR, Luan P, Wroblewski VJ. Quantitation of hepcidin from human and mouse serum using liquid chromatography tandem mass spectrometry. *Blood* 2007;110:1048-54.
6. Tanno T, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT, Moroney JW, Reed CH, Luban NL, Wang RH, Eling TE, Childs R, Ganz T, Leitman SF, Fucharoen S, Miller JL. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. *Nat Med* 2007;13:1096-101.
7. Mast AE, Blinder MA, Gronowski AM, Chumley C, Scott MG. Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. *Clin Chem* 1998;44:45-51.
8. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. *Annu Rev Nutr* 2006;26:323-42.
9. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, Dawkins FW, Acton RT, Harris EL, Gordeuk VR, Leiendecker-Foster C, Speechley M, Snively BM, Holup JL, Thomson E, Sholinsky P. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med* 2005;352:1769-78.
10. Steinberg KK, Cogswell ME, Chang JC, Caudill SP, McQuillan GM, Bowman BA, Grummer-Strawn LM, Sampson EJ, Khoury MJ, Gallagher ML. Prevalence of C282Y and H63D mutations in the hemochromatosis (HFE) gene in the United States. *JAMA* 2001;285:2216-22.
11. Barry E, Derhammer T, Elsea SH. Prevalence of three hereditary hemochromatosis mutant alleles in the Michigan Caucasian population. *Community Genet* 2005;8:173-9.
12. Finch CA, Cook JD, Labbe RF, Culala M. Effect of blood donation on iron stores as evaluated by serum ferritin. *Blood* 1977;50:441-7.
13. Simon TL, Garry PJ, Hooper EM. Iron stores in blood donors. *JAMA* 1981;245:2038-43.
14. Simon TL, Hunt WC, Garry PJ. Iron supplementation for menstruating female blood donors. *Transfusion* 1984;24:469-72.
15. Bianco C, Brittenham G, Gilcher RO, Gordeuk VR, Kushner JP, Sayers M, Chambers L, Counts RB, Aylesworth C, Nemo G, Alving B. Maintaining iron balance in women blood donors of childbearing age: summary of a workshop. *Transfusion* 2002;42:798-805.
16. Newman B. Iron depletion by whole-blood donation harms menstruating females: the current whole-blood-

- collection paradigm needs to be changed. *Transfusion* 2006;46:1667-81.
17. Cafolla A, Dragoni F, Girelli G, Tosti ME, Costante A, Pastorelli D, Bedogni G, Scott S. Folate status in Italian blood donors: relation to gender and smoking. *Haematologica* 2000;85:694-8.
18. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004;306:2090-3.
19. Finch C. Regulators of iron balance in humans. *Blood* 1994;84:1697-702.
20. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. *Blood* 2006;108:3730-5.
21. Piperno A, Girelli D, Nemeth E, Trombini P, Bozzini C, Poggiali E, Phung Y, Ganz T, Camaschella C. Blunted hepcidin response to oral iron challenge in HFE-related hemochromatosis. *Blood* 2007;110:4096-100. 