Supplemental Methods

REDS-II Participants.

Six blood centers participated in the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II): Blood Center of Wisconsin (Milwaukee, WI); the American Red Cross New England Region (Dedham, MA); Hoxworth Blood Center/University of Cincinnati Academic Health Center (Cincinnati, OH); American Red Cross Southern Region (Douglasville, GA); Blood Centers of the Pacific (San Francisco, CA); and the Institute for Transfusion Medicine (Pittsburgh, PA). The REDS-II coordinating center was Westat (Rockville, MD) and Blood Systems Research Institute (San Francisco, CA) served as the REDS-II Central Laboratory.

Statistical Analyses.

Descriptive analysis included frequencies of demographic and donation characteristics. In addition, scatter plots were developed to show the correlation between log10 ferritin and log10 hepcidin values at each visit for all donors. Profile plots were also created to look at the change in venous hemoglobin, log10 ferritin and log10 hepcidin longitudinally.

To directly measure the longitudinal effects (due to both donation intensity and recovery time), multivariable repeated measures regression models were developed for venous hemoglobin, log10 scale ferritin, and log 10 scale hepcidin. The venous hemoglobin and ferritin models were the same as those developed earlier[1] but are provided here for convenience. The previous analyses examined the change in venous hemoglobin and ferritin, whereas this current analysis focused on 1) the change in hepcidin as a result of repeat blood donations and 2) the effect of ferritin and changes in hepcidin on venous hemoglobin after controlling for selected covariates. The models included longitudinal effects for donation variables that changed at each donation (i.e. dynamic variables); recovery time measured as "time since last donation (in weeks)" and donation intensity measured as "number of donations in the past

24 months." Additional donation variables included race/ethnicity, gender, age, weight, smoking, iron supplementation, menstrual status, pregnancy history, *HFE* genotype (C282Y and H63D),[2] G277S transferrin genotype,[3] and blood center. The models also included age-by-gender and weight-by-gender as interaction terms.

In a supplemental hemoglobin model, the additional effects of ferritin and hepcidin were estimated using a two-stage model. By two-stage we mean that selected parameters at the second stage were held fixed to values calculated at the first stage. Specifically the first stage model was the multivariable repeated measures regression model described above with venous hemoglobin as the outcome. The motivation for using this two stage approach was that the first stage parameter estimates drew from a larger data set of all 2,425 blood donors enrolled in RISE, and thus would derive more reliable parameter estimates. The second stage model was conducted using data from the 148 blood donors with hepcidin measures (114 first-time females and 34 frequent males with 880 donation visits).

At the second stage, we introduced a single variable that classified donors based on their hepcidin and ferritin measurements. For a given visit, a hepcidin measurement was available at that visit as well as at the previous one. We classified each hepcidin measurement into high or low based on a cut-off of 45.7 ng/ml. This hepcidin cut-off represents approximately the lower 15% of normal males and approximately the lower 30% of normal females.[4] Sequential visits were then classified as having either 1) "low or decreasing hepcidin" or 2) "high or increasing hepcidin". In the first instance, hepcidin was consistently low between two sequential visits or was high at the first visit and dropped down to a low level at the second visit; both patterns show evidence of a persistent systemic response to iron deficiency. For sequential visits classified as "high or increasing hepcidin", hepcidin was consistently high between visits or moved from low to high. A visit was classified as having low ferritin if the plasma ferritin measurement was $\leq$26 ng/ml, which corresponds to the bottom 2.5% of first time/reactivated male donors

enrolled in RISE at baseline.[5,6] The combination of the hepcidin trend and plasma ferritin was used to define a single hepcidin/ferritin variable, the four levels of which are included in the results section.

All analyses were done using SAS (SAS 9.2 (2008) SAS Institute Inc, Cary NC).

References

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Supplemental Table 1: Demographic and other characteristics of enrolled blood donors (n=148)

	First-Time Females Donors N (%)	Repeat Male Donors* N (%)
Geometric Mean Ferritin (range) (ng/ml)	46.8 (6.0 – 457.1)	11.0 (3.0 – 72.4)
Geometric Mean Hepcidin (range) (ng/ml)	91.2 (11.8 – 269.2)	25.7 (0.2 – 104.7)
Mean Hemoglobin (range) (g/dl)	13.8 (11.9 – 16.1)	13.6 (11.8 – 16.1)
Mean RBC count (range) ($x10^6$ cells/μL)	4.5 (3.7 – 5.0)	4.8 (3.9 – 6.0)
Mean Reticulocyte count (range) ($x10^3$ cells/μL)	25.6 (0.0 – 98.9)	40.8 (0.0 – 139.5)
Total	114	34
Race/Ethnicity		
Asian	0 (0.0)	0 (0.0)
Black	1 (0.9)	0 (0.0)
Hispanic	2 (1.8)	0 (0.0)
White	110 (96.5)	33 (97.1)
Other	1 (0.9)	1 (2.9)
Age (in years)		
16 – 29	12 (10.5)	1 (2.9)
30 – 39	19 (16.7)	0 (0.0)
40 – 49	24 (21.0)	3 (8.8)
50 – 59	35 (30.7)	15 (44.1)
60+	24 (21.0)	15 (44.1)
***HFE* genotype**		
Wild Type	72 (63.2)	19 (55.9)
Heterozygous H63D	30 (26.3)	9 (26.5)
Heterozygous C282Y	9 (7.9)	6 (17.6)
Double Mutation †	3 (2.6)	0 (0.0)
Blood Center		
A	49 (43.0)	7 (20.6)
B	—	—
C	—	—
D	16 (14.0)	4 (11.8)
E	31 (27.2)	16 (47.0)

F	18 (15.8)	7 (20.6)
Smoking		
Current smoker	11 (9.6)	1 (2.9)
Past/Non/Unknown	103 (90.4)	33 (97.1)
Weight (in kg)		
< 68	40 (35.1)	0 (0.0)
68 – 78	40 (35.1)	8 (23.5)
79 – 90	16 (14.0)	15 (44.1)
91 or greater	18 (15.8)	11 (32.4)
Iron Supplementation		
Iron Supplement	55 (48.2)	12 (35.3)
No iron Supplement	59 (51.8)	22 (64.7)
Pregnancy Status (females)		
Ever Pregnant	88 (77.2)	—
Unknown pregnancy status	0 (0.0)	—
Never pregnant	26 (23.8)	—
Menstrual Cycle (females)		
Periods have stopped	54 (47.4)	—
Still having periods	60 (52.6)	—
G277S Genotype		
Wild Type	94 (82.4)	28 (82.4)
Hetero- or Homozygous	19 (16.7)	6 (17.6)
Missing	1 (0.9)	0 (0.0)
Red Cell donations in previous 2 years		
First-Time/Reactivated Donors	114 (100.0)	—
≤ 3 donations	—	3 (8.8)
4-9 donations	—	21 (61.8)
10+ donations	—	10 (29.4)

* Repeat male donors have been deferred for low hemoglobin

† “Double Mutation” refers to any combination of the 3 mutations