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This supplementary material has been provided by the authors to give readers additional information about their work.

e Methods

Calculation of Time to 80% Hemoglobin Recovery

Time to 80% hemoglobin recovery was estimated by fitting to the data a regression model estimating hemoglobin as a function of time since donation. Parameter estimates from the fitted model provided the basis for estimating recovery time. We chose to establish a primary endpoint of time to 80% rather than 100% hemoglobin recovery because hemoglobin recovery occurs at a rate that decelerates as equilibrium is approached, resulting in a plot of hemoglobin vs time that rises and then flattens. Estimates of time to a point of the flattened part of the curve are much less reliable than estimates of time to a point at which the curve is rising more steeply. In addition, analytical variability in hemoglobin levels (a single baseline measurement was used to determine pre-donation hemoglobin value) is +/- 2-3% day-to-day. [1] Therefore, to ensure that estimates of recovery were less affected by variability in the measurement of baseline and recovered hemoglobin, reversal of 80% of the post-donation decrease in hemoglobin, rather than 100% was considered "recovery".

The hypothetical plot of hemoglobin vs. time in e Supplement Figure 1 below illustrates recovery of hemoglobin after red cell donation. Time=0 represents the time of donation; thus, negative values on the time scale represent days before the donation and positive values represent days after the donation. Points in the plot represent hypothetical hemoglobin measurements. In this example, the donor is assumed to be in equilibrium prior to donation, as illustrated by the horizontal line segment. The variability of points around the line segment and the recovery curve represents the combination of biological variation and measurement error. The circled point is h_0, the level of hemoglobin immediately before donation. In this example, the value is above the donor's pre-donation equilibrium (but this value could also be below equilibrium). The recovery curve is described by the equation, $h_t = \beta_1 - \beta_2 e^{-\beta_3 t}$, where h_t is hemoglobin at time t, and β_1, β_2 and β_3 are model parameters. The equation was derived by assuming that the rate of recovery at any point after donation was a function of the difference between equilibrium hemoglobin and current hemoglobin: $\delta h_t / \delta t = \beta_3(\beta_1 - h_t)$. β_1 is hemoglobin at equilibrium after recovery, β_2 is β_1 minus hemoglobin at time zero on the recovery curve (the y axis intercept), and β_3 is a parameter that captures the rate of recovery. $\beta_1 - \beta_2$ should not be interpreted as the actual measured hemoglobin immediately after donation because this value does not allow for post-donation volume equilibration and was not measured in HEIRS. To allow for volume equilibration with the real data, we treated hemoglobin on day 3 as representing the true post-donation level. With this approach, the difference between hemoglobin at the post-donation nadir and at the post-donation equilibrium would be $\beta_1 - \left(\beta_1 - \beta_2 e^{-3\beta_3}\right) = \beta_2 e^{-3\beta_3}$. In effect, we would be assuming a 3-day lag before measurable recovery begins. However, a parameter for the lag is not included in the theoretical model illustrated in Figure 1. Because we did not measure hemoglobin during the three day lag, a lag parameter was not needed for model fitting and would not alter our estimates of recovery time measured from time 0. In other words, a lag parameter is not needed for model fitting and adding this parameter would not alter our estimates of recovery time or the confidence limits for it.

In this example, hemoglobin returns to the pre-donation level after donation but this need not be the case. As the results from HEIRS demonstrate, the post-donation equilibrium value, B_1 may be close to, above or below pre-donation hemoglobin, depending on pre-donation iron stores and whether a donor took iron during recovery. The rate parameter, B_3 varied similarly. For example, participants who reached a post-donation equilibrium above pre-donation hemoglobin also had recovery curves that rose faster than the curves for participants who reached equilibrium at lower hemoglobin levels.

To estimate time to 80% recovery, we first estimated the hemoglobin level at 80% recovery and then determined the time required to reach that level. Hemoglobin at 80% recovery was calculated as $h_3+0.80*(h_0-h_3) = 0.8h_0+0.2h_3$, where h_3 is the hemoglobin 3 days after donation, calculated from the recovery model, and h_0 is the observed predonation hemoglobin.

Time to 80% recovery was calculated by rearranging the recovery equation as

$$t_{rec} = \frac{-1}{\beta_3}\log_e\left(\frac{\beta_1 - (0.8h_0 + 0.2h_3)}{\beta_2}\right)$$

Estimating Population Average Hemoglobin Recovery Time vs Hemoglobin Recovery Time for Individual Participants

The model fitting approach described above was applied to the seven visit-specific mean hemoglobin levels in each of the four groups that were considered in the analysis. In effect, this provided an estimate of mean recovery time within that group. The bootstrap confidence limits are confidence limits for mean recovery time; i.e. they are not estimates of the dispersion of individual recovery times. Recognizing the importance of assessing the distribution of individual recovery times, we attempted to apply the modeling approach above to estimate recovery time for each participant. However, the software for fitting the model failed to produce reliable parameter estimates for 14 of the 193 participants (7%) who had completed at least 6 of 7 planned follow up visits. (Participants who completed fewer than 6 visits were not included in the primary analysis. The majority of the excluded participants failed to give reliable parameter estimates for recovery time; many had too few observations to permit fitting a 3-parameter model at all.) The main reasons for failure to give reliable parameter estimates were flat recovery curves. These 14 participants were concentrated within specific groups, primarily lower ferritin participants not taking iron. Given the differences in mean recovery times among groups, we were concerned that excluding these cases would bias the overall distribution of recovery times, leading to possible misinterpretation of the results. These problems were not encountered when we modeled the visit-specific means. Therefore, our primary analysis was to obtain reliable estimates of population average recovery time.

eDiscussion

In the hemoglobin recovery study conducted by Pottgiesser, et al., investigators chose a mixed end point consisting of either recovery of hemoglobin mass to 98% of baseline <u>or</u> recovery to a plateau "equilibrium value," if an individual did not recover to 98% of baseline.[2] Their use of 98% as a recovery end point is similar to our endpoint of 80% recovery of the hemoglobin drop post-donation - since our average post-donation drop was 10% of baseline. However, in our opinion, Pottgiesser, et al.'s approach (i.e. alternative post-donation equilibrium as a recovery endpoint) when the equilibrium is below pre-donation hemoglobin level may lead to underestimation of the time to hemoglobin recovery. This is illustrated in Figure 2B of our paper where the equilibrium recovery in non-supplemented higher ferritin group participants falls below baseline; in contrast, the equilibrium recovery exceeds baseline in participants on iron. Given the failure of mean hemoglobin in some participants not on iron to return to pre-donation levels in our study, we elected not to apply the post donation equilibrium approach to our data. Instead, cases in which the 80% recovery value was not achieved at the end of the 168 day observation period were assigned a recovery time of >168 days. We believe this difference in analytical approach, possibly along with the differences in the methodology (total body hemoglobin v. hemoglobin concentration) and the donor population studied, as discussed in the manuscript, may explain the faster recovery times reported by Pottgiesser, et al. None-the-less, the highest ferritin quartile subgroup (upper horizontal line in Figure 4 in the manuscript) most comparable to the Pottgiesser participants(with mean ferritin 76.7 ng/ml in HEIRS v. 60.4 ng/ml in the latter) had the fastest hemoglobin recovery in the non-supplemented arm, averaging 54 days v. 36 days in Pottgiesser, et al.

References

1. Dot D, Miro J, Fuentes-Anderiu X. Within-subject biological variation of hematological quantities and analytical goals. Arch Pathol Lab Med. 1992; 116:825-26.

2. Pottgiesser T, Specker W, Umhau M, Dickhuth HH, Roecker K, Schumacher YO. Recovery of hemoglobin mass after blood donation. Transfusion 2008 ;48:1390-7.

eTable. Post-donation decline in hemoglobin in treatment, gender, and iron status groups

treatment	baseline					visit 1					difference				
	mean	sd	n	lower CL	upper CL	mean	sd	n	lower CL	upper CL	mean	sd	n	lower CL	upper CL
iron	13.71	1.12	96	13.49	13.93	12.38	1.2	96	12.14	12.62	-1.33	0.63	96	-1.46	-1.20
no iron	13.94	1.25	97	13.69	14.19	12.52	1.39	95	12.24	12.80	-1.4	0.72	95	-1.54	-1.26

gender	baseline					visit 1					difference				
	mean	sd	n	lower CL	upper CL	mean	sd	n	lower CL	upper CL	mean	sd	n	lower CL	upper CL
men	14.57	1.21	75	14.30	14.84	13.47	1.21	73	13.19	13.75	-1.09	0.62	73	-1.23	-0.95
women	13.36	0.91	118	13.20	13.52	11.82	0.89	118	11.66	11.98	-1.54	0.66	118	-1.66	-1.42

ferritin	baseline					visit 1					difference				
	mean	sd	n	lower CL	upper CL	mean	sd	n	lower CL	upper CL	mean	sd	n	lower CL	upper CL
<=26	13.4	1.11	95	13.18	13.62	12.01	1.22	94	11.76	12.26	-1.36	0.7	94	-1.50	-1.22
>26	14.24	1.12	98	14.02	14.46	12.88	1.23	97	12.64	13.12	-1.38	0.66	97	-1.51	-1.25

Baseline and post-donation hemoglobin levels, and the absolute decrease in hemoglobin levels (baseline to post donation, with 95% CIs) for the iron and no iron groups overall, for men and women, and for the low ferritin and higher ferritin groups. Post-donation hemoglobin is comparable in all groups except for a significantly greater decrease in post-donation hemoglobin in women compared to men, attributed to blood volume differences.

**eFigure 1. Idealized Hemoglobin Recovery Curve Following Blood Donation**
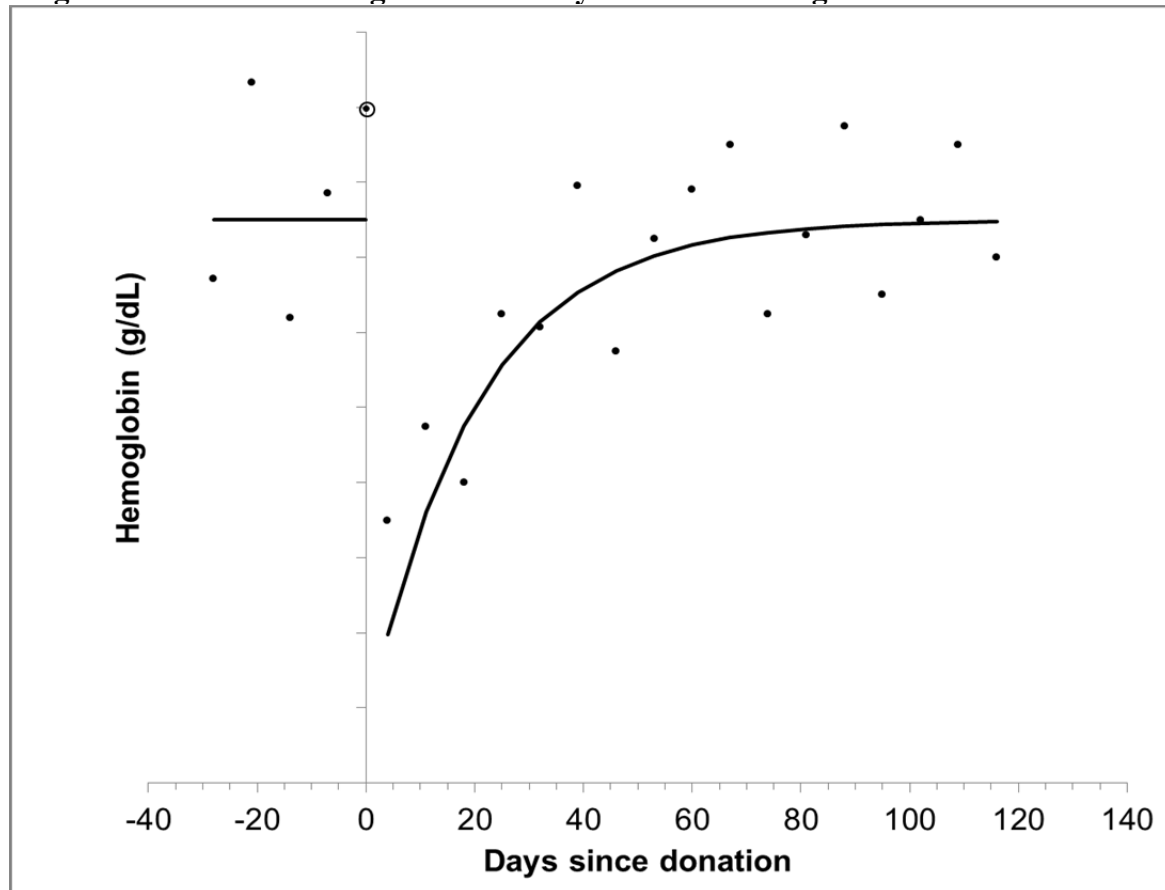
The hypothetical plot of hemoglobin vs. time illustrates recovery of hemoglobin after red cell donation. Time=0 represents the time of donation. Negative values on the time scale represent days before the donation and positive values represent days after the donation. Points in the plot represent hypothetical hemoglobin measurements. In this example, the donor is assumed to be in equilibrium prior to donation, as illustrated by the horizontal line segment. The variability of points around the line segment and the recovery curve represents the combination of biological variation and measurement error. The circled point is h_0, the level of hemoglobin immediately before donation. In this example, the value is above the donor's pre-donation equilibrium (but this value could also be below equilibrium). The recovery curve is described by the equation $h_t = \beta_1 - \beta_2 e^{-\beta_3 t}$, where h_t is hemoglobin at time t, β_1 is hemoglobin at equilibrium after recovery, $\beta_1 - \beta_2$ is hemoglobin at time zero on the recovery curve and β_3 is a parameter that captures the rate of recovery (see text).

eFigure 2. Hemoglobin recovery to 12.5 g/dl in men and women

A. Low ferritin (< 26 ng/ml) at baseline
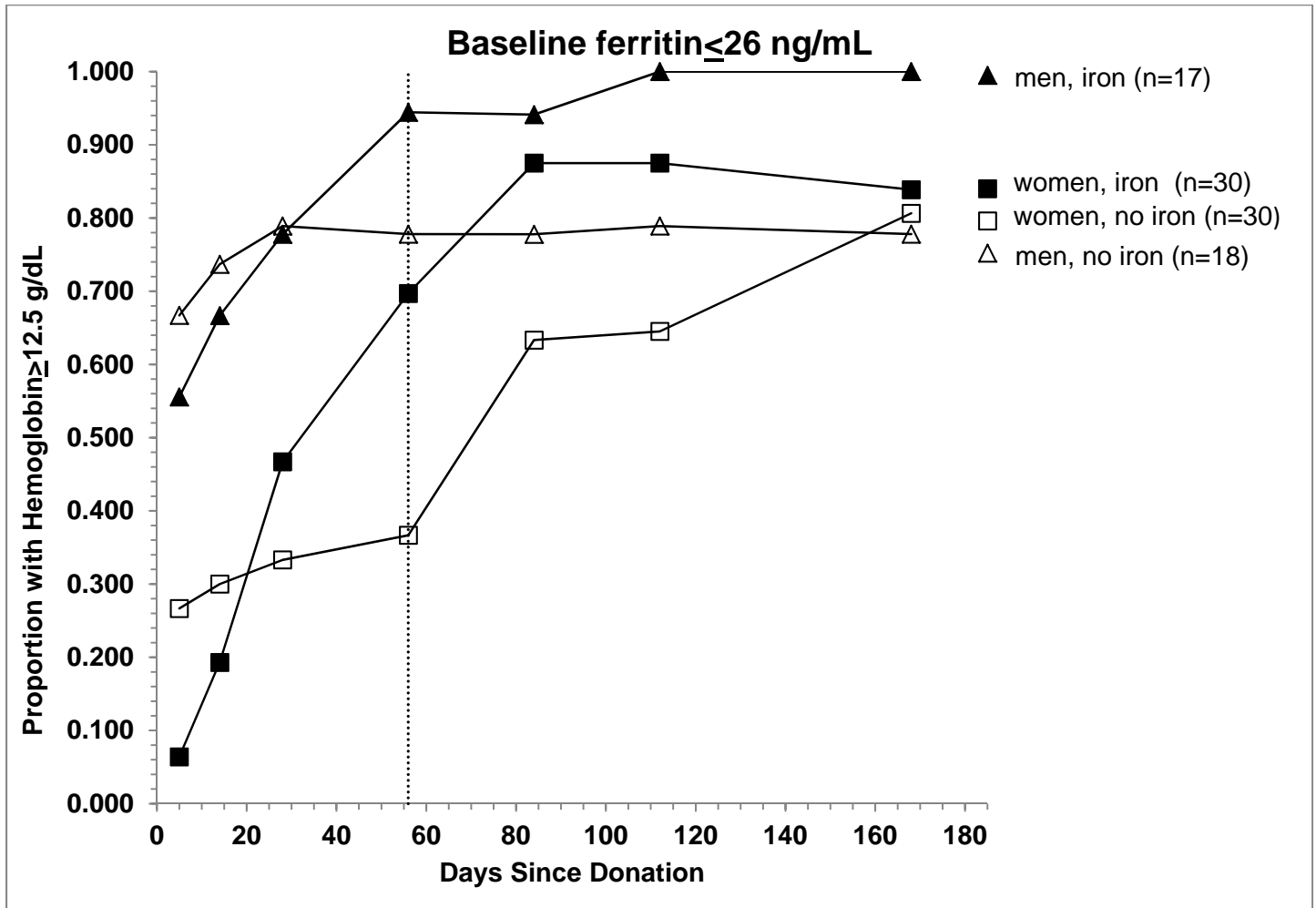
B. Higher ferritin (> 26 ng/ml) at baseline
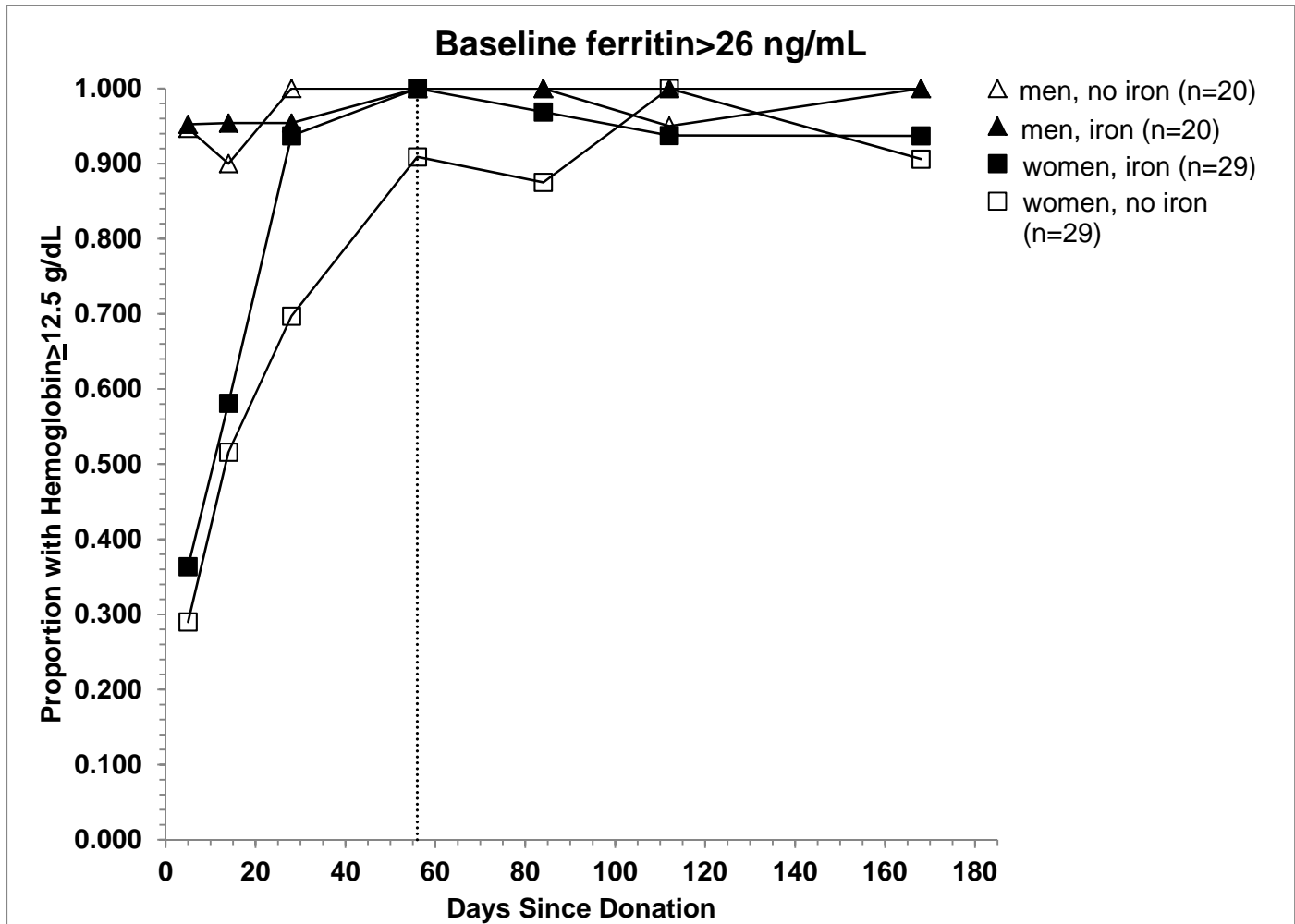
Hemoglobin recovery (venous) to 12.5 gm/dl is shown as a proportion of each group over time. A vertical line indicates 56 days from the time of donation A. Low ferritin (< 26 ng/ml). B. Higher ferritin (>26 ng/ml). Number in each subgroup shown in parentheses.