

# **Hemoglobin and Iron Recovery Study (HEIRS)**

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**For the NHLBI Recipient Epidemiology and Donor Evaluation Study-III (REDS-III)**

## **2. Concept Synopsis and Study Schema**

This is a REDS-III pilot intervention clinical trial to directly assess recovery times of hemoglobin and recovery of iron stores following whole blood donation. The study will assess the effects of iron supplementation, pre-donation iron stores, gender and older age on hemoglobin recovery. 400 donors will be recruited for screening after withdrawal of 500 ml whole blood, across a range of prior blood donation intensities and associated iron stores; 200 donors will be selected among the 400 screened donors, based on their ferritin values, and will be serially studied. In order to ensure representation of older donors (who have lower hemoglobin levels and blunted erythropoietic reserves) specific efforts will be made to enroll donors over 60 years old.

Following consent, a successful whole blood donation, and determination of appropriate iron stores, donors will be randomized to 38 mg per day of iron or not, to be continued for 24 weeks of follow up. Randomization will be stratified on gender, age (<60 vs  $\geq$ 60 years) and iron status (ferritin  $\leq$ 26 vs >26). Samples for hemoglobin and ferritin will be obtained pre-donation (from the sample pouch) and at seven follow-up intervals from 3-7 days post donation to 24 weeks. Soluble transferrin receptor (sTfR) will be obtained at baseline and the final draw only. Reticulocyte counts and indices will be obtained in a representative subset.

The primary objective will be to determine the time-course of short-term hemoglobin recovery in iron-depleted donors on iron supplementation compared to unsupplemented controls. Secondary objectives will be to determine time to hemoglobin recovery and iron stores in relation to pre-donation iron status, gender, and older donor age. The study will also provide an assessment of the feasibility, compliance rate, and side effects related to daily iron supplementation over a longer time interval (24 weeks) than contemporary iron replacement trials in which iron is typically given for only 60 days after blood donation.

## \*Recovery of Hemoglobin Schema

Recruit 400 repeat blood donors  $\geq 18$  yo:

- No donations for 4 months
- Successful WB donation Time 0
- Not taking – or willing to discontinue – iron containing supplements
- Recruitment target for  $>60$  yo will be 20% of enrollment.

- Consent/enrollment
- Retention tubes collected – Hb, retic (one center only), and rapid ferritin determined
- Time 0 plasma sample saved for use for those donors selected to continue

- Return for Day 3-7 visit. Project 350 to return.
- If rapid ferritin was  $\leq 26$  ng/ml: Full samples collected day 3-7 and also use Day 0 samples (Hemoglobin, ferritin, sTfR,  $\pm$ hepcidin). Donor continues in study. Randomized to receive iron pills (Fe gluconate: 38mg iron) or not. Project approximately 30% = 100 are iron depleted: 50 in each RX arm
- If rapid ferritin  $>26$ : Donor is NOT iron depleted – Donors are recruited to continue or are dismissed if relevant recruitment strata are already complete (See protocol). Project 250 will be iron replete; Select 100 to continue within the stratification scheme: Randomized to receive iron pills (Fe gluconate: 38mg iron) or not.
- Donors continuing get full sample collection on Day 3-7 and also use samples from Day 0 (Hemoglobin, ferritin, sTfR,  $\pm$ hepcidin).
- For 150 dismissed donors, samples for Hb only are collected at day 3-7 visit to better assess expected post donation Hb drop.

- 200 donors (100 iron depleted; 100 iron replete) return for 2,4,8,12,16, and 24 week samples. 24 week sample can be combined with a blood donation.
- Full sample collection (Hemoglobin (retics at one center only), ferritin, sTfR,  $\pm$ hepcidin)
- For donors assigned to iron supplements, compliance will be assessed and new iron pills dispensed.

- Hemoglobin determined after each visit.
- Frozen plasma samples retained for end-of-study batch testing.

Estimated compliance and budget assumptions both groups:

- For conservative budget estimate: 80% will complete all visits to 24 weeks. 20% will drop out before the week 8 visit (before the 4 follow-up visits required for three point regression analysis). Batch analysis for ferritin and sTfR will be performed at the end only on the 80% completing donors.
- For Power calculation at least 50% will continue to final recovery visit. In Iron depleted group at least 25 iron supplemented and 25 controls will complete study. See Power analysis.
- Screening and follow-up sample analysis will be Hgb and ferritin only. For donors completing study, Day 0 and final visits will also assay sTfR.

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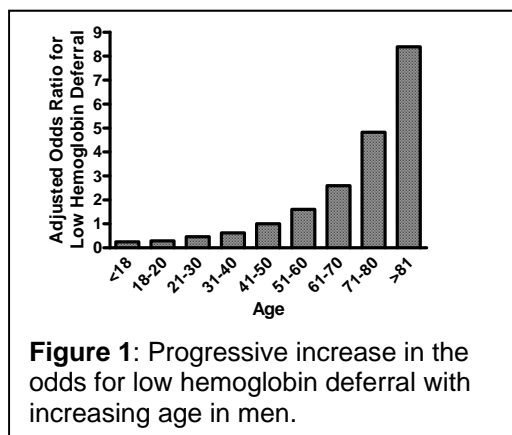
## Organization of Protocol

### 4.1. Background and Significance

There has been limited investigation of the short term events involving restoration of hemoglobin and iron stores following whole blood donation using modern analytical techniques. Classic articles from the 1940's and 50's showed that recovery of hemoglobin took 21-28 days in healthy male and female volunteers with a peak of reticulocytes about 9 days after donation[1], but that return to pre-donation hemoglobin took longer in established blood donors – mean 49.6 days, with 26% requiring over 8 weeks. Addition of iron salts resulted in faster regeneration of hemoglobin to 5 weeks.[2]

Using a sensitive carbon monoxide rebreathing method to precisely quantify hemoglobin mass, German investigators found that the total amount of hemoglobin lost after donation of 550 ml whole blood was replenished in 36 +/- 11 days (range 20-59 days) in a group of young (30 +/- 10 years) iron replete (mean ferritin 60.4 ug/L) male volunteers.[3] However, in the recently completed RISE study of “frequent” donors, interdonation intervals at less than 14 weeks were found to be associated with increased risk of low hemoglobin deferral (OR ~2-2.5) and iron deficiency (OR ~3-4.4 for ferritin < 12ug/L).[4] ORs for hemoglobin deferral became non-significant at 14 weeks, suggesting that about 3-4 months is required for the bone marrow to replenish lost red cells, likely limited by the rate of gastrointestinal absorption of iron.[5] Interestingly, an interval of approximately 120 days (the average lifespan of RBCs) is the expected recovery period if no net increase in hematopoietic activity (red cell production rate) were to occur.[1] Moreover, recent data from over 4 million donations in the REDSII donation database indicate that hemoglobin recovery time appears to be longer even in “non-frequent” regular blood donors, attaining baseline levels at approximately 14 weeks after the previous donation. [Spencer BR, RISE Analysis Group]

While iron status is clearly a key determinant in hemoglobin regeneration, other demographic factors such as older age may also be important. In epidemiologic studies performed over the last decade there is evidence of increasing iron deficiency and hemoglobin deferral in blood donors as a whole [6] as well as demographic shifts in donor age.



In the RISE study 9.9% of the donors were deferred for low hemoglobin and 77% of these donors were iron deficient. The importance of low hemoglobin deferral in older donors is demonstrated by results from a study of over 715,000 donors performed using the REDS-II donation deferral database.[7] This study found that the odds for low hemoglobin deferral progressively increases with age in men to the point where those between 61 and 70 years old have 10 times higher odds for deferral than men under 20 years old, while men between 71 and 80 years old have 19 times higher odds, and those 81 and older have 33 times higher odds (**Figure 1**). In women, this effect of aging is muted because of menstrual blood loss in younger women. Yet, similar to men, there are progressively

increasing odds for deferral in women over 60 years old. Importantly, post-menopausal women only have slightly lower odds for low hemoglobin deferral than menstruating women, the demographic group in which most studies of low hemoglobin deferral have been performed. [8,9]

Anemia is more prevalent in older individuals, present in about 10% of those over 65 years of age and in about 20% of those over 85 years of age suggesting an overall decrease in effective erythropoiesis that occurs with aging.[10] While there are a number of potential causes of anemia, of particular note for otherwise healthy older blood donors is the increased prevalence of inflammatory diseases such as arthritis, as well as the increased use of medicines, such as antacids and H2 receptor blockers, which decrease iron absorption. [11] This proposal will examine changes in the physiological responses to blood donation that occur with aging, particularly in regard to iron absorption and maintenance of an adequate hemoglobin level and how iron replacement therapy may improve hemoglobin recovery and iron stores in this growing demographic segment. Donors > 60 years old contributed 16.6% of the blood units collected in the REDSII donor database. Collection of blood from older donors will become increasingly important as the population ages. However, there currently is a dramatic decrease in the number of donations by donors over 60 years old. Very little research has focused on how to retain otherwise healthy older individuals as successful donors as they age. This is similar to a general trend in other areas of medicine where older individuals are under-represented in clinical studies.[12,13]

Proposals to change the inter-donation interval, hemoglobin donation cut-off, or implement iron supplementation would be informed with new studies of the short-term response following whole blood donation, in donors of varying iron status and age segment, with or without concurrent iron supplementation. For example, if the current 56 day US interdonation interval were to be lengthened for routine donor management, evidence of satisfactory hemoglobin recovery in iron-replete donors may justify using the 56 day standard in donors qualified by ferritin (or similar) testing. Likewise, evidence of satisfactory hemoglobin recovery in iron-depleted donors taking iron may allow using the shorter interval in iron-supplemented donors. This study will also allow an assessment of the replenishment of iron stores at various levels of iron depletion and the effect of supplemental iron. In addition, since exogenous iron and erythropoietin have been shown to facilitate repetitive autologous red cell donations [14] it is possible but not yet demonstrated that iron supplementation may enhance the erythropoietic response, even in donors who are iron replete. If verified, this would also have important implications for iron supplementation programs.

## **4.2. Objectives**

### **4.2.1. Primary Objective**

- 4.2.1.1.** To determine the hemoglobin recovery time in iron depleted donors on iron supplementation compared to unsupplemented iron depleted controls.

### **4.2.2. Secondary Objectives**

- 4.2.2.1.** To determine hemoglobin recovery time in relation to pre-donation iron status in unsupplemented iron depleted and iron replete donors.
- 4.2.2.2.** To determine the hemoglobin recovery time in iron replete donors on iron supplementation compared to unsupplemented iron replete controls.

- 4.2.2.3. To assess the effect of gender and age on hemoglobin recovery time, after correcting for iron status.
- 4.2.2.4. To assess differences in the recovery of iron stores based on gender and older age with- and without iron supplementation
- 4.2.2.5. To provide pilot data on the duration of daily 38 mg iron supplementation required to restore hemoglobin and iron to pre-donation baseline levels
- 4.2.2.6. To provide pilot data on donor compliance and side effects of daily continuous iron supplementation

### **4.3. Study Population of Specimens for Analyses**

#### **4.3.1. Inclusion Criteria**

Eligible male and female repeat blood donors will be recruited. Eligible donors must have acceptable hemoglobin value ( $\geq 12.5$  gm/dl). An effort will be made to enroll all age groups  $\geq 18$  years but a specific recruitment target for older donors  $> 60$  years will be 20% of enrollment. An effort to enroll donors across the spectrum of donation intensity in the period 4-24 months prior to enrollment will be made. In order to ensure a sufficient number of donors with iron deficiency, recruitment efforts among the donation intensity strata (see Section 4) may be adjusted as the study progresses.

#### **4.3.2. Exclusion Criteria**

Donors who are taking iron supplements, including multivitamins containing iron will be asked to discontinue taking them for the 24 week duration of the study or will not be eligible if they are unwilling to do this. Donors must not have donated any blood component for at least four months (to assist in allowing adequate restoration of hemoglobin to near baseline) and agree not to donate any additional blood component for 24 weeks. They can donate if otherwise eligible when they report for their 24 week final visit. Donors who are deferred for low hemoglobin or any other reason will be excluded.

### **4.4. Study Enrollment or Specimen Procurement**

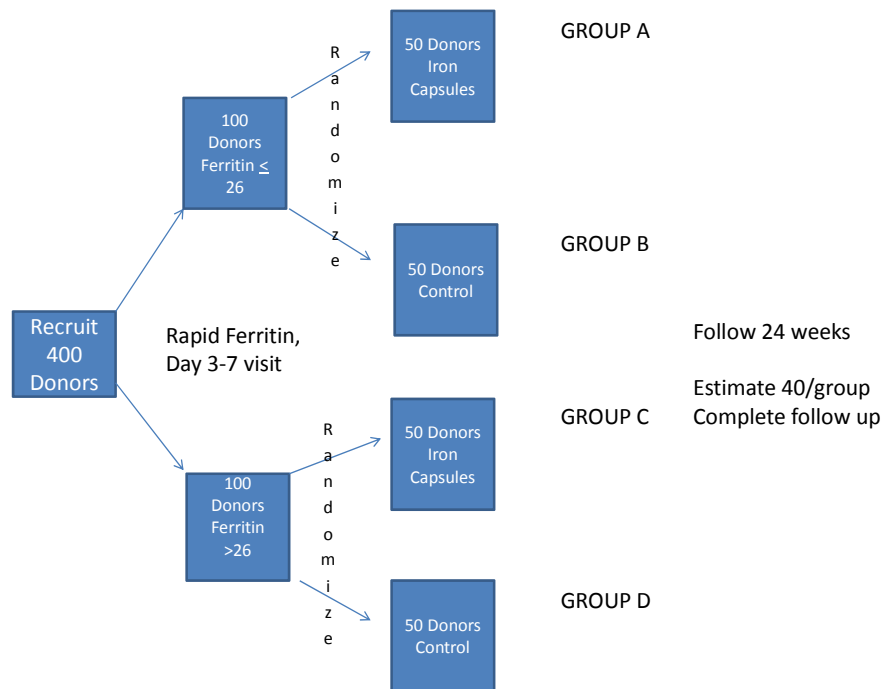
#### **4.4.1. Screening/Recruitment/Specimen Acquisition**

Men and women who have not donated in the past 4 months will be identified through blood center donor databases and recruited by letter or at blood drives to participate in the study. Screening for # donations in last 2 years will be performed to enrich the recruitment of donors who are likely to be iron deficient ( $> 1$  donation in the last year). Donors donating more frequently will be targeted in order to enroll those with a greater chance of being iron depleted. Those responding to the letter will be asked to visit the blood center to provide informed consent. Only donors who successfully donate a unit of whole blood at the visit will be enrolled. Enrolled donors will have agreed to stop taking multiple vitamins and/or iron supplements other than those provided during the course of the study.

After informed consent to participate in the study is obtained, a rapid turnaround ferritin assay on an aliquot from the retention tube will be performed. Based on this assay, at the 3-7 day post-donation visit donors will be categorized as iron depleted (plasma ferritin  $\leq 26$  ug/L) or not. All iron depleted donors (estimated as 100 subjects or approximately 30% of enrollees) and 100 of the remaining 250 subjects, randomly selected as strata are filled by age and gender, will be randomized to receive a daily capsule of ferrous gluconate containing 38 mg of elemental iron (50 in each group) while the other 50 in each group will serve as untreated (not blinded) controls for the duration of the study. After obtaining the day 3-7 sample for hemoglobin, to gather more information on short term fluid and hemoglobin changes after blood donation, the other 150 iron replete donors' will be dismissed and their participation in the study will end.

Although we expect iron supplementation to have the greatest therapeutic effect on hemoglobin recovery in iron-depleted donors, hemoglobin recovery may also be improved by iron supplements in non-iron depleted donors. Many blood donors are just above the 26 ng/ml ferritin threshold and donation of a unit of blood results in the loss of ~230 mg iron, enough to decrease ferritin levels by 20-30 ng/ml. In addition, absent bone marrow iron stores and iron deficient erythropoiesis may be seen with ferritin levels as high as 40 ng/ml [14a]. Even if one is iron-replete, taking a 38 mg iron supplement daily for a limited time after loss of 230 mg from donation is unlikely to result in a marked elevation in ferritin (possibly up to 50 ng/ml above baseline, assuming maximal absorption of 4 mg/day for 24 weeks) and this would not result in clinical toxicity.

Figure 2. Study Enrollment (See Figure 3 for detail on stratification)



All selected donors will donate a unit of WB at time 0, and samples will be obtained for analysis at times 0 (pre-donation), 3-7 days, and for continuing donors 2, 4, 8, 12, 16, and



24 weeks. The iron capsules for the selected donors will be continued for the duration of the follow up period. Donors will not donate any blood component again until completion of the study, except that the 24 week sample can be collected from a subsequent blood donation.

At each time point peripheral blood samples will be collected for analyses to determine the post-donation restoration time for hemoglobin and key parameters of iron metabolism. It is anticipated that all testing can be performed using 7 ml of blood per time point (56 ml total) which totals about 12% of the 500 ml of blood removed during whole blood donation and should not meaningfully affect interpretation of results. Hemoglobin recovery will be considered to have occurred when 80% of the original hemoglobin drop (based on the difference between the baseline and the 3-7 day sample) is achieved. While recovery to pre-donation levels would seem a desirable endpoint, time to 100% recovery can be very difficult to determine precisely. Plots of post-donation hemoglobin vs. time since donation tend to form rising curves that flatten out as donors return to pre-donation levels. The shallow slope at the upper end reduces the precision of estimates of the time to a given hemoglobin level. The problem is further complicated by measurement error and biological fluctuations in hemoglobin, which can make it difficult to determine when the 100% return point is reached. A more precise estimate of time to a specified level can be obtained if we consider time to a point somewhat farther down the curve. For these reasons we have chosen 80% recovery as our target. We believe that it is reasonable to assume that a person who reaches 80% recovery faster will also reach 100% recovery faster.

#### **4.4.2. Operational Feasibility:**

Recruitment of donors will be constrained by two opposing requirements for previous donation history. First, we need to recruit donors who are likely to be iron depleted. To achieve the estimated 30% iron depleted status, donors will need to be regular donors with  $\geq 1$  red cell donation in the last 12 months. (Based on RISE, donors who give  $\geq 2$  donations (female) or  $\geq 3$  donations (male) are likely to be 40-60% iron depleted – there is less reliable information on the iron status of less frequent repeat donors.) Second, donors cannot have donated for at least 4 months so as to ensure near complete recovery of hemoglobin from preceding blood donations. Since the recovery time of hemoglobin is a key outcome of this study, the follow-up of donors after the index whole blood donation is for 24 weeks, not 4 months, as in the waiting period for enrollment, to ensure adequate estimates of the recovery time of the slower recovering donors. It is possible that the 4 month pre-donation waiting period is not long enough, but the difficulty of ensuring an iron depleted donor population (frequent previous donations) conflicts with longer proposed waiting intervals. This possibility will be assessed, in part by analysis of the recovery time in models in which the previous donation interval is included as a variable.

Although specific donor recruitment targets for previous donation frequency will not be attempted, tracking of recruitment at each center and by the coordinating center will be used to ensure a reasonable spread of donors across iron store levels. Iron tablets containing 38 mg elemental iron are used in STRIDE and will be dispensed by a central pharmacy located at The Blood Center of Wisconsin (Dr Alan Mast is the PI for STRIDE).

Compliance in the iron supplement group will be assessed by pill counts at each visit. Compliance of subjects randomized to iron or no iron will also be assessed by interview at each visit to determine deviation from assigned treatment including crossover effects (donors not taking required iron and/or donors assigned to no iron taking OTC iron supplement). An independent medical monitor will review adverse event reports for all participating sites. Donors on iron with complaints will be managed by either reducing the frequency to every other day (mild symptoms), temporary discontinuation then restarting with reduced frequency to every other day, or dropping out of the study (significant symptoms or troubling symptoms with reduced frequency).

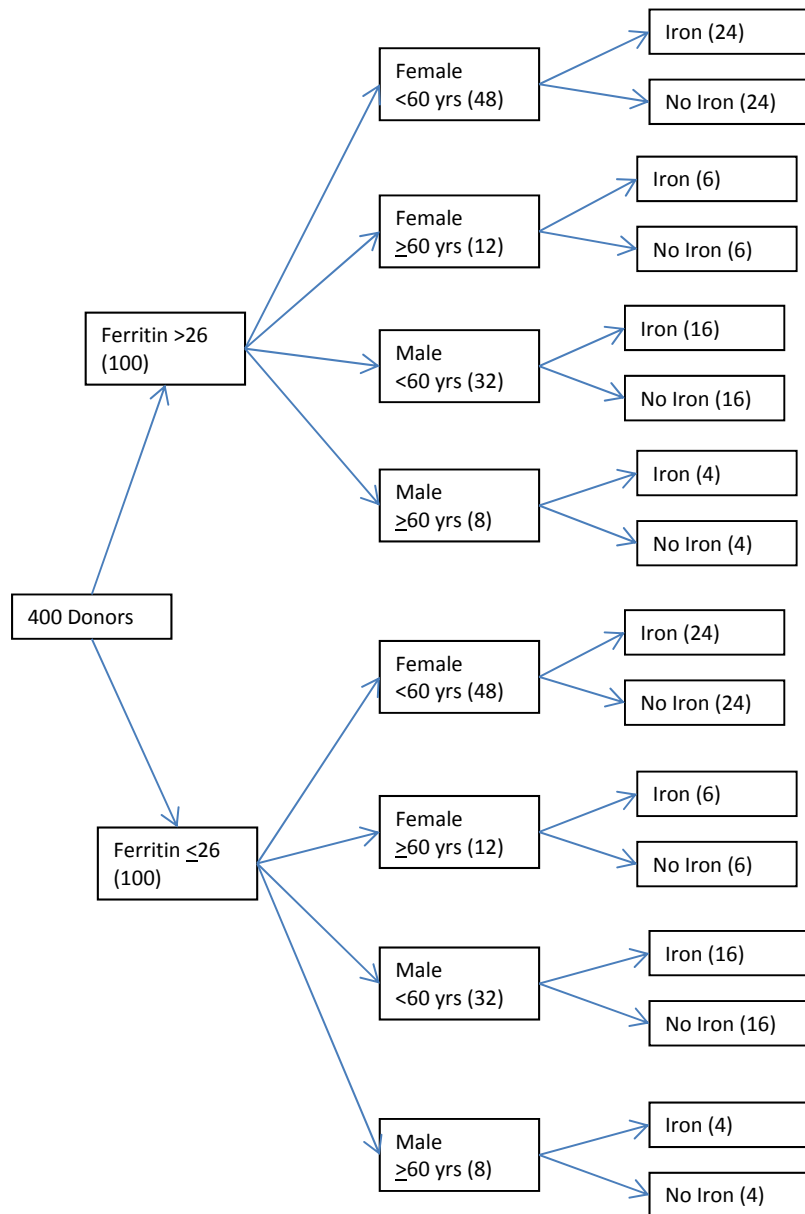
#### 4.4.3. Stratification at Randomization

Randomization to iron/no iron, with equal allocation to the two treatment arms, will be performed but placebos and blinding will not be employed. A placebo arm would also add complexity and cost in a “fast-track” study primarily intended to determine hemoglobin and iron recovery and not efficacy of iron per se as in other ongoing studies such as STRIDE-Study to Reduce Iron Deficiency (randomized controlled trial with 3 treatment arms that compare 60 days of 38 mg elemental iron, 19 mg elemental iron, and placebo after each blood donation). We do not believe that lack of a placebo will bias study results because the endpoints are based on objective laboratory measurements.

Randomization will be stratified on gender and age (<60 vs. ≥60 years) as well as iron status. To ensure that iron status, gender and age are balanced across treatment groups, randomization will take place within the 8 strata defined by the 2X2X2 cross-classification of the three variables (see Figure 3). As noted in Section 4.4.1, we will randomize equal numbers of iron replete and iron depleted donors. We will not attempt to randomize equal numbers of younger or older men or women within either iron status category. Given the distribution of age and gender among blood donors and the limited time available, recruiting equal numbers is not feasible. Based on prior experience, we expect to recruit the numbers shown in parentheses in Figure 3. Initially, all eligible subjects who agree to participate will be recruited for the study. We will monitor enrollment to ensure that the expected smaller groups have at least the representation listed in Figure 3. Recruitment in a stratum can be halted once the planned number of study subjects has been randomized. Recruitment in the other strata will continue until the planned number in each is reached. We will adjust our recruitment strategy half way through the recruitment process if recruitment in the smaller groups does not meet the expected goal.

Because participation will require multiple visits for samples and, for half the donors, daily iron supplements are required, donor payment is recommended. Donors can be consented after their time 0 blood donation, assuming retention tubes are available. Otherwise recruitment after eligibility is determined will be necessary.

Figure 3. Stratified Randomization. Numbers in ( ) reflect expected accrual based on 60% female/40% male enrollment.



Because participation will require multiple visits for samples and, for half the donors, daily iron supplements are required, donor payment is recommended. Donors can be consented after their time 0 blood donation, assuming retention tubes are available. Otherwise recruitment after eligibility is determined will be necessary.

#### 4.5. Interventions (if applicable)

##### 4.5.1. Preparation

The treatment group will receive a capsule containing ferrous gluconate (38 mg of elemental iron) for 24 weeks.

#### 4.5.2. Administration (e.g., schedule, allowable modifications, where applied, etc)

Iron will be taken daily, and modified if necessary to every other day. Compliance and total dose taken will be recorded at each visit.

#### 4.5.3. Control Population

The control group will consist of untreated “standard of care” group followed for 24 weeks (a placebo will not be used).

### 4.6. Measurement

#### 4.6.1. Schedule of Measurement

Donors will donate a unit of WB at time 0, and peripheral blood samples will be obtained for analysis at times 0 (pre-donation), 3-7 days, and (if selected) 2, 4, 8, 12, 16, and 24 weeks.

#### 4.6.2. Definitions (as appropriate)

Iron-depleted: Ferritin  $\leq$  26 ug/L  
Iron-replete: Ferritin  $>$  26 ug/L

#### 4.6.3. Assessment and Measurement Procedures

**CBC** will be performed to assess the **hemoglobin**, the key parameter for assessing the erythropoietic response following blood donation. This testing needs to be completed in less than 24 hours following obtaining the sample and will be performed locally using automated hematology instruments widely available in blood center and hospital laboratories.

**Reticulocyte** results will not be required for determination of hemoglobin or iron recovery. Nevertheless, reticulocyte analysis is of interest and desired in the protocol, particularly at earlier time points, as it is a direct measure of erythropoiesis. However, because blood center hematology analyzers may not be able to perform reticulocyte analysis in a cost effective manner (or at all), acquisition of this analyte will likely require use of outside hospital labs. The protocol team evaluated several options for this analyte, including limiting it to selected donors and/or centers, and decided to perform it only for donors at ITXM.

**Ferritin** is the single best test for assessing total body iron stores, however, its value is limited because it is an acute phase reactant and may be falsely elevated in donors with arthritis or other inflammatory disease.[15] However, in a donor population ferritin is considered a reliable overall indicator of iron status. This testing will be performed on frozen plasma samples on all serial bleeds, batched and sent to a central laboratory for analysis.

**Soluble transferrin receptor (sTfR)** (first & last samples only) is a very sensitive test for iron deficiency that is not affected by inflammatory disease. Determination of the ratio of log (sTfR/ferritin) has been shown to be the most sensitive and specific means for diagnosis of iron deficiency using peripheral blood tests using methodology developed by Cook and co-workers.[16] This testing will be performed on frozen plasma samples

on the baseline donation and final samples only, batched and sent to a central laboratory for analysis. Where ferritin and sTfR analyses are not concordant, the donor may be assessed for inflammatory conditions that may artefactually raise ferritin. If confirmed such donors would be censored.

**Total body iron** will be calculated at baseline and final visit and iron balance (including absorption) described in all longitudinally-followed donors after donation. Use of the two reciprocally regulated measurements in blood donor studies indicates high sensitivity in the detection of iron depletion. [16a] Ferritin measurements (which reflect storage iron) and sTfR values (which reflect functional iron) are combined into a ratio,  $\log(\text{sTfR}/\text{ferritin})$ , as a derived measurement to Total Body Iron using the formula:

$$\text{Total Body Iron (mg/kg)} = - [\log(\text{sTfR}/\text{ferritin}) - 2.8229]/0.1207$$

The investigators estimated average tissue iron stores of  $9.82 \pm 2.82$  mg/kg ( $776 \pm 313$  mg) in men and  $4.87 \pm 4.14$  mg/kg ( $309 \pm 346$  mg) in women. Use of this formula will allow us to accurately quantify iron stores (and iron deficit) in study subjects and examine how they change in the different intervention arms of the proposed study. A major advantage of directly quantifying iron stores is that it will allow accurate assessment of the impact of iron supplementation and provide a clear picture of iron homeostasis. We will measure the amount of storage iron present at baseline and at the end of the study to assess how much of the deficit is replenished. We will also explore the variability of absorption rates according to the degree of iron storage repletion, gender, and age.

**Hepcidin** is a recently identified iron regulatory hormone that prevents dietary iron absorption and release of iron from macrophages. [17] High intensity blood donors have very low hepcidin levels.[18] Since hepcidin is an acute phase reactant, a proportion of older donors with underlying inflammatory disease are expected to have increased hepcidin that will limit their ability to absorb dietary iron and recycle macrophage iron stores for new red blood cell synthesis. Plasma hepcidin testing is available for clinical research studies through Intrinsic LifeSciences in San Diego,CA. [19] Frozen plasma samples will be batched and sent for analysis if funds or other support can be obtained for this purpose.

**Time to hemoglobin recovery** will be determined from the serial hemoglobin measurements using a regression method that is described in Section 4.9.

#### 4.6.4. Specimen collection procedures

At baseline, a 4-7 ml EDTA sample will be obtained from the pre-collection sample pouch. Either this tube or an additional 2-3 ml tube will be used for CBC and reticulocyte analysis (local lab, result w/i 24 hrs) and 5 ml centrifuged to obtain plasma. Overall sample loss will be limited to 7 mL per time point. Plasma will aliquoted into two 1 ml plastic vials and frozen for batch testing. Ferritin/sTfR will be done by ARUP labs and hepcidin sample will be frozen and stored. Hepcidin and other iron studies that might be needed after initial data analysis may take up to 2 years from collection to complete. The frozen samples will be retained by centers until this testing is complete, and then

discarded. There are no plans for repository storage. No genetic studies or studies unrelated to iron stores will be performed on the frozen samples. Samples will be obtained by venipuncture at the follow up time points. They will be aliquoted, tested and/or and stored in the same fashion.

#### 4.6.5. Special test procedures if required

Not applicable.

#### 4.7. Survey Considerations and OMB Requirements

A survey is not planned as a part of this study.

#### 4.8. Data Management

The DCC will provide a data management system for the study. The system will include facilities to capture data from the domestic hubs and from the central laboratory. Data from the central lab will be obtained by upload of bulk files of assay results using a protocol that will satisfy the security requirements of REDS-III. The format of the files, such as excel files, SAS datasets or formatted text files will be determined jointly by personnel from the DCC and central laboratory. Two approaches to capturing data from the domestic hubs are under consideration. One would make use of a web-based data entry system. With this approach, the sites would enter data directly into the secure environment at the DCC using web-based access. The other would involve a study management system (SMS) under which data would be entered into files at the hubs and the files would periodically be uploaded to the DCC using a secure transfer system like the one to be employed by the central lab. The DCC would be responsible for developing and distributing the SMS.

See Section 7. , Appendices, for data elements.

#### 4.9. Statistical Considerations

With observations at intervals of 2 weeks or longer, we are unlikely to observe the actual time to hemoglobin recovery. A reasonably precise measure of recovery time can be obtained by modeling hemoglobin (h) as a function of time (t) for an individual and then finding the time point at which the fitted curve crosses the recovery boundary. The specific model will be determined by examining the data. One candidate model is  $h = \theta_1 - \theta_2(\exp(-\theta_3 t))$  where  $\exp$  is the base of natural logarithms and  $-\theta_3 t$  is an exponent. To estimate recovery time, we first determine the hemoglobin level at the recovery boundary, which is the hemoglobin level representing 80% recovery to the pre-donation state. The hemoglobin value at the recovery point is  $h_{rec} = h_{pre} - 0.2(h_{pre} - h_{post})$  where  $h_{rec}$  is hemoglobin at recovery,  $h_{pre}$  is pre-donation hemoglobin and  $h_{post}$  is hemoglobin 3-7 days after donation. Time to  $h_{rec}$  is

$$t_{rec} = (-1/\theta_3) \log_e[(\theta_1 - h_{rec})/\theta_2]$$

where  $\log_e$  refers to natural logarithms. We will treat  $t_{\text{rec}}$  as the outcome variable in determining the effect of iron supplementation on time to recovery.

#### 4.9.1. Hypothesized outcome rate and smallest difference to detect w/high statistical power

Sample size calculations were based primarily on the comparison of recovery times in iron-depleted donors randomized to iron supplementation or no supplementation. Hemoglobin recovery time on iron supplementation was assumed to be similar to recovery time in iron replete donors as reported by Pottgiesser et al (Transfusion 2008;48:1390-97). Thus, mean recovery time on iron supplementation was assumed to be 5 weeks. This was extended to 7 weeks to allow for some lack of compliance with treatment because of side effects of iron supplements. We will consider iron supplementation in iron-depleted donors to be of strong clinical interest if the supplements cut mean recovery time in half; i.e. from 14 to 7 weeks. In practice, we expect an even stronger effect as average recovery time without the supplements is likely to exceed 14 weeks.

#### 4.9.2. Sample size and power

Sample size calculations were based on the following assumptions, in addition to the assumptions about mean recovery time stated above:

- 1) Following Pottgiesser et al, it was assumed that there will be a 3-week lag between donation and the first observed recovery in each treatment group. The lag needs to be taken into account in fitting the statistical model for power calculations.
- 2) Recovery times in the two groups were assumed to be exponentially distributed after subtracting the 3-week lag.
- 3) Subjects will be followed for a maximum of 24 weeks.

Monte Carlo simulations and logrank statistics were employed for the sample size calculations. Based on the assumptions above, 18 subjects per treatment group will provide power of 0.80 and 24 subjects per group will provide power of 0.90 when the mean recovery times are 7 and 14 weeks.

There are some points to consider in evaluating the sample size calculations. First, it is highly unlikely that recovery times in either arm will be exponentially distributed. The study population will be heterogeneous with respect to iron levels at study entry and this heterogeneity will likely affect the distribution of recovery times. Other factors, such as post-donation behavior, will likely also affect the recovery times. The end result is that recovery times will depart from the assumed exponential distribution. However, the departures from an exponential distribution could actually increase statistical power if the benefits of iron supplementation are greater in the more depleted subjects.

The assumption of a 3-week lag in time to first observed recovery in both treatment arms may also be incorrect. The lag may be shorter in those randomized to receive iron than in those randomized not to receive iron. This, however, would probably increase statistical power.

While the sample size calculations have been adjusted for some noncompliance with iron supplementation, they have not been adjusted for other problems. For example, there is no adjustment for loss to follow-up. Subjects who are lost to follow-up can be treated as right censored at the point of loss if they have not recovered by that time and the number of measurements of hemoglobin to that point is not sufficient to project time to recovery. Neither is there any adjustment for crossover. Subjects could stop taking iron owing to side effects or those in the control arm could start taking over the counter medications that contain iron. Either event could reduce the size of the treatment effect in the study. For all of these reasons, the sample size was increased to 50 iron depleted donors per treatment arm.

#### **4.9.3. Analytic Approach (primary, secondary and subgroup analyses)**

Proportional hazards regression will be employed to model recovery time ( $t_{rec}$ ) as a function of treatment assignment (Group A v. B, and Group A v. C, iron supplement or control; Also Group B v. D, unsupplemented iron depleted v. unsupplemented iron-replete). Subjects with recovery times that exceed 24 weeks will be treated at right censored at 24 weeks. Dropouts will be treated as censored at the last observation. The same method will be used for secondary analyses to determine the effects of age and gender on recovery time. We may be able to determine whether the effect of iron supplementation on recovery time varies between age groups or genders by adding interactions between treatment assignment and the other predictors to the model – these, however, are secondary objectives. Graphical displays of recovery times for the various subgroups (treatment groups, genders, age groups) will be produced as Kaplan-Meier plots. We will also examine the effects of pre-donation iron status on recovery time by treating the measures of pre-donation iron stores as continuous predictors of time to recovery. This analysis will include the iron depleted and iron replete subjects who did not receive supplemental iron. We will examine the effects of iron stores on recovery graphically by dividing the subjects in the analysis into subsets, such as quartiles of pre-donation iron and producing Kaplan-Meier plots of time to recovery.

#### **4.10. Human Subjects**

Three of the four REDS-III centers are involved in STRIDE and have direct experience in conducting an iron supplementation protocol. Procedures used in STRIDE, such as side effect reporting and medical monitoring, will be incorporated into this study (see Appendix 3, Data Safety and Monitoring Plan). The present study will enroll male and female donors whose race/ethnicity is representative of the general donor population in at REDS-III centers. The risk of providing iron to donors with hemochromatosis will be addressed in the protocol by excluding donors with baseline ferritin over 300 ug/L. The risk of masking GI malignancy is considered remote because this is a short term study and donors must be generally healthy to qualify for blood donation. In addition, donors will have at least 4 months in which to regenerate their baseline hematologic status from the previous blood donation and any donor who has low hemoglobin deferral at enrollment assessment (that could be due to GI malignancy) will not be entered in the study. There is also the risk of adverse effects of iron therapy, which are mainly gastrointestinal irritation. The dose of iron to be used is considered to be extremely safe



with minimal side effects. Pills will be packaged in individual blister packs to minimize accidental poisoning of children. There is also the possibility of release of information collected as part of the survey or laboratory tests. Human subject information collected in this study will be strictly confidential and will be protected by unique study ID, maintaining identifiers only in the blood center, and a certificate of confidentiality. Subjects will be informed in the Consent that they may withdraw from the study at any time but that the retained samples and study data up to that point will still be used by the study. They will be further informed: "We want you to understand this provision because the study is limited in the number of donors that can be recruited, and the study will invest considerable time and resources in collecting sequential samples from each donor. Loss of already collected samples and data could jeopardize the objectives of the study." Following completion of data analysis, the de-identified study data will be prepared as a public data set to be made available to other researchers according to NIH guidelines.

Benefits to the subjects are related to those who receive iron therapy. Such subjects will be more likely to recover hemoglobin levels and iron stores rapidly. It is believed that the benefits outweigh the minimal risks associated with the proposed study.

#### **4.11. Timeline**

### **5. References**

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## 6. Appendices

### Appendix 1. Donor Data Elements

Name

Donor ID (From Donor data base)

Informed Consent signed and on file

Subject ID (Protocol specific)

Gender

Age

Height

Weight

Race/Ethnicity

Whole Blood Number of Enrollment WB donation

Date of Enrollment Donation (Time = 0)

FS Hb/Hct at enrollment donation

Date of previous donation

Date of previous red cell donation (Usually the same)

Previous red cell donation type (WB, 2RBC, RBC pheresis)

Number of red cell donations in past two years (WB=1; 2RBC =2; RBC pheresis =1)

Iron supplement at time of enrollment? (Yes/No – Open field for description if Yes)

Iron supplements discontinued? (Must be “Yes”)

Enrollment ferritin

## Appendix 2. Sample Data Elements

Donor id

Visit # (0 = enrollment; 1-7)

Date

Sufficient volume?

Appropriate processing and storage?

Ferritin (ng/mL) – Visits 0 and 1; Visit 2-7, groups A-D

sTfR (mg/L) – First and last Visits only, groups A-D

Venous Hemoglobin (g/dL) – Visits 0 and 1; Visit 2-7, groups A-D

MVC (fL), at ITXM only

MCHC (g/dL), at ITXM only

MCH (pg??), at ITXM only

Reticulocytes (#/uL) – Visits 0 and 1; Visit 2-7, groups A-D, at ITXM only

## **Appendix 3. Data and safety monitoring plan (DSMP)**

### **1. Adverse Event Criteria and Reporting**

#### **Reporting Adverse Events.**

Reporting requirements are calibrated to the seriousness of the event and the perceived relationship to the study intervention (Ferrous gluconate). For this study the reporting requirements will be based on the type and severity of adverse event using the descriptive terminology developed by the National Cancer Institute for use in reporting adverse events: Common Toxicology Criteria for Adverse Events (CTCAE) version 4.0, dated May 29, 2009. The website for the CTCAE is [http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/docs/ctcae4.pdf](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae4.pdf). The CTCAE includes a grading (severity) scale for each adverse event term. Grades were developed using the following guidelines:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

#### **Definitions**

Adverse Event (AE) – Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Serious Adverse Event (SAE) – Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria: results in death; is life-threatening (places the subject at immediate risk of death from the event as it occurs); requires inpatient hospitalization or prolongation of existing hospitalization; results in a persistent or significant disability/incapacity; results in a congenital anomaly/birth defect; or any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Grade 4 and 5 events will always be considered Serious Adverse Events. Many Grade 3 events and some Grade 1 and 2 events may meet the definition of a Serious Adverse Event.

Unexpected Adverse Event – Any adverse event occurring in one or more subjects in a research protocol, the nature, severity, or frequency of which is not consistent with

either: the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol–related documents, such as the IRB-approved research protocol or the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts; or the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject’s predisposing risk factor profile for the adverse event.

Unanticipated problem involving risks to subjects or others (UP): Any incident, experience, or outcome that meets all of the following criteria: unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; related or possibly related to a subject’s participation in the research; and suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

Attribution – the determination of whether an adverse event is related to a medical treatment procedure.

Attribution categories:

1. Not Related - Event clearly related to other factors (e.g., clinical state, other therapies; concomitant drugs)
2. Possibly Related - Sequence of event is compatible with study drug, device, or procedure, but could have been produced by other factors
3. Probably Related - Sequence of event is compatible with study drug, device, or procedure and cannot be explained by other factors without much doubt
4. Definitely Related - Sequence of event is compatible with study drug, device, or procedure and beyond doubt cannot be explained by other factors

### **Types of Adverse Events to be reported in the Hemoglobin Recovery study**

Participants in the Hemoglobin Recovery Study who are taking iron tablets (ferrous gluconate containing 38 mg elemental iron) may encounter side effects and symptoms associated with the tablets. Based on past experience most of the reported side effects are expected to be Grade 1 or 2. The following guide is to provide information to the site PI and the Safety Medical Monitor (see below).

Common expected symptoms associated with ferrous gluconate are:

- Black Stool<sup>1</sup>
- Constipation<sup>2</sup>
- Abdominal Cramping
- Diarrhea

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<sup>1</sup> Dark/black stools are common and to be expected. This is not a reason for the participant to stop taking the pills and de-enroll.

<sup>2</sup> Some mild or moderate constipation can be alleviated with increasing water intake. Along with the standard responses, recommend that the participant adds 3 or 4 glasses of water to their normal daily intake; one with each meal.

- Nausea
- Vomiting
- Bloating/Indigestion
- Headache
- Metallic Taste/Sore Mouth
- Hyperactive Bowel Sounds (loud grumbling sounds)
- Itching/Rash/Hives
- Leg Cramps

For the purpose of this study, any single symptom or combination of the symptoms above that persist without improvement for more than 3 days, and result(s) in emergency department care, and/ or hospitalization, or any Unanticipated Problem regardless of duration or medical treatment, will be reported immediately (within 24 hours) to the Safety Medical Monitor for expedited review.

### **Review of Events for Subjects Enrolled in the Hemoglobin Recovery study**

The DCC will contract with a Safety Medical Monitor (SMM) to review adverse events. The Observational Safety Monitoring Board (OSMB) is an independent board appointed by the NHLBI. The principal role of the OSMB is to regularly monitor the data from the study, review and assess the performance of its operations, and make recommendations, as appropriate, to the NHLBI.

The Safety Medical Monitor and the NHLBI will be notified immediately when a serious Adverse Event probably or definitely related to the ferrous gluconate tablets or an event resulting in death (regardless of attribution) is reported to the DCC. The Safety Medical Monitor will review the event as soon as the materials are available. Following their review, the Medical Monitor will complete a form summarizing his/her findings.

Every two months the DCC will send NHLBI summary information about all adverse events reported.

## **2. Potential Risks and Benefits for Participants**

Potential risks include

- Adverse effects of iron therapy, which is mainly gastrointestinal irritation.
- The possibility of iron therapy masking gastrointestinal bleeding
- Release of information collected as part of the survey or laboratory tests.

Benefits to the subjects are related to those who receive iron therapy. Such subjects will be more likely to recover hemoglobin level faster and will be less likely to remain iron deficient. It is believed that the benefits outweigh the minimal risks associated with the proposed study.

## **3. Protection against Study Risks**

The study investigators are aware of the possibility of iron therapy masking gastrointestinal bleeding in study subjects. Surveillance for gastrointestinal blood loss will be accomplished as follows:

### **3.1 Informed Consent Process:**

- The consent form will clearly identify a potential risk for use of iron supplements in masking of anemia developing secondary to gastrointestinal blood loss that could result from a variety of disorders including colon cancer.
- The consent form will inform subjects that it is recommended by the US Preventative Services Task force that all men and women over 50 years old be screened for colon cancer by their personal physician (Ann Int Med 2008, 149:627-638).
- The consent form will inform subjects that anyone with a family history of colon cancer should have discussions with their personal physician for recommendations for how they should be screened for the disease.

### **3.2 Safety Monitoring**

We propose to have a Safety Medical Monitor (SMM):

- This individual should be a physician who is independent of the protocol committee, e.g., a consultant to RTI
- The SMM will have access to all study laboratory data and blood donation data. Adverse Event data will be sent on an ongoing (real time) basis.
- The SMM will monitor the iron supplementation intervention arm of the study for unintended consequences, adverse events and any other safety concerns.
- The SMM will be particularly aware of the potential for iron supplementation to mask gastrointestinal blood loss and the potential to increase ferritin levels. He/she will monitor subjects for unexpected changes in ferritin (as determined at end of study) and will advise study investigators if he/she determines there is a need for specific medical care for individual study subjects.

### **3.3 Study Design**

All donors will be screened for potential iron overload and those with ferritin  $\geq 300$  ng/ml will not be allowed to enroll in the proposed study. The study is designed to provide donors with oral iron to be taken daily for 24 weeks after a single blood donation. The SMM will make medical recommendations on an individual basis to the study investigators about the type of medical care that might be needed for subjects experiencing adverse events,

### **3.4 Adverse Event Recording and Monitoring Plan**

The PI at each participating center will be informed of serious adverse events as soon as they occur and will notify the SMM within 24 hours of notification. We will record and monitor potential adverse drug reactions. These events will be summarized in monthly reports and discussed regularly in team meetings. Institutional Review Boards (IRBs) for all participating institutions will be kept appropriately informed about any adverse events.



#### **4. Protection of Confidentiality**

The potential for a breach in confidentiality is minimal given the steps that will be taken to maintain study participants' confidentiality. All donor records will be maintained in secure files at participating institutions. Information on donors enrolled in the proposed study will be identified by a unique donor number for the purposes of transmission to RTI or for laboratory testing.