BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-056316
Article Type:	Protocol
Date Submitted by the Author:	10-Aug-2021
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Keywords:	Blood bank & transfusion medicine < HAEMATOLOGY, Anaemia < HAEMATOLOGY, Clinical trials < THERAPEUTICS

Ferritin-guided iron supplementation in whole blood donors: Optimal dosage, donor Response, return, and Efficacy (FORTE) – a randomized controlled trial protocol

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Protocol version 1, 10-08-2021

Word count: 3760

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Abstract

Background: Frequent whole blood donors have an increased risk of developing iron deficiency. Iron deficiency can have detrimental health effects when left untreated. Donation intervals are commonly too short to replenish iron stores, and extending these reduces donor availability. Oral iron supplementation is known to shorten iron store recovery time but may also induce gastrointestinal complaints. We aim to optimize the effectiveness of iron supplements while minimizing the risks of side effects. Therefore, we will evaluate the impact of different iron supplementation protocols in terms of dosage and frequency on ferritin and hemoglobin levels, gastrointestinal side-effects, iron deficiency-related symptoms, and donor return compared to placebo supplementation.

Methods: Twelve hundred whole blood donors with ferritin levels ≤30 µg/L are included into a double-blind, randomized controlled trial. Participants are randomly allocated to one of six arms, administering capsules containing 0, 30, or 60 mg of iron, either on alternate days or daily for 56 days. At baseline and 56, 122, and 182 days of follow-up, ferritin and hemoglobin levels are measured, and compliance, donor return, dietary iron intake, gastrointestinal, iron deficiency-related symptoms, and general health are assessed by questionnaire.

Significance and outlook: This study will provide a comprehensive overview of the effects of different frequencies and dosages of administration of iron supplements on iron status and health effects, thereby considering individual differences in treatment adherence and lifestyle. The outcome will provide scientific evidence to guide the debate if and how oral iron supplements may support the recovery of whole blood donors with low ferritin levels.

Trial registration: The Dutch trial registry NL8590.

Keywords: Blood donation; Ferritin; Iron Deficiency; Iron Supplementation; Donor Health

Strengths and limitations of this study

- This is a large (n = 1200), double-blind, randomized controlled trial to determine the optimal iron supplementation protocol in terms of both intake frequency and dosage.
- Outcome variables include ferritin and hemoglobin levels, complete blood counts, iron deficiency-related symptoms, and gastrointestinal side effects.
- Participants are thoroughly characterized at baseline regarding social-economic status, medical background, physical fitness, dietary intake, and smoking status.
- A limitation is the limited number of follow-up visits; however, these time points are the most relevant, and temporal patterns are modeled based on earlier studies.

Introduction

Due to the hemoglobin (Hb)-bound iron loss during donation, regular whole blood donors are prone to developing iron deficiency, often in the absence of anemia[1,2]. Iron deficiency without anemia is characterized by reduced serum ferritin levels. It is highly prevalent amongst frequent whole blood donors, with 15.0% of the female and 9.4% of male Dutch donors having ferritin levels <15 µg/L [3]. Reduced ferritin levels increase the risk of developing anemia and are associated with iron deficiency-related symptoms, including fatigue, reduced exercise endurance, restless legs, PICA (appetite for non-nutritious substances), and reduced neurocognitive functioning[4-8].

At most international blood banks, including the Netherlands, Hb levels are measured before donation to safeguard donor health and blood product quality[9]. However, Hb levels do not reflect the amount of stored iron, which is significantly impacted by whole blood donations[10]. Therefore, ferritin measurements have become more common amongst blood banks to assess the donor's iron storage. Sanquin incorporated ferritin-guided donation intervals, deferring donors for 6 or 12 months when ferritin levels are ≥15 and ≤30 µg/L or <15 µg/L, respectively. These cut-off values are based on WHO standards, as described previously[3]. However, donor deferral has been shown to demoralize donors and reduce donor return rates [3,11,12].

Several studies have shown that oral iron supplementation reduces the post-donation recovery time of ferritin and Hb levels to pre-donation levels[13,14]. While donors in the countries like the United States, Finland, and Denmark are already advised about iron supplementation for iron storage recovery after donation, other blood services are hesitant, often due to ethical concerns[15-17]. Kiss *et al.* showed in a randomized controlled trial that post-donation iron supplementation led to full recovery of ferritin levels within 56 days, whereas the non-iron supplementing group did not reach full recovery after 160 days [18].

In several clinical trials, the iron supplement ferrous bisglycinate has been shown to result in a relatively high fractional iron uptake. This has been attributed to its iron-bound chelates that prevent the iron from binding to other dietary compounds (e.g., tannins, catechols, and phytates) that inhibit iron absorption[19-22]. Furthermore, supplementation with ferrous bisglycinate has been shown to lower the risk of gastrointestinal discomfort compared to other iron formulations, possibly due to the increased uptake by intestinal mucosal cells, leading to less iron entering the colon[19,23,24]. Therefore, ferrous bisglycinate is already used by the Danish blood bank for donors who suffer from gastrointestinal side-effects[25,26].

Iron absorption in the duodenum and its entry into the plasma compartment is regulated by the peptide hormone hepcidin[27]. It has been shown that iron supplementation leads to an increased hepatic production of hepcidin in iron-depleted women, causing a reduced intestinal iron uptake up to 24 hours post-supplementation[28,29]. While the dosages of elemental iron used in previous studies range from 19 to 240 mg/day, lower dosed iron supplements are shown effective in recovering the iron stores post-donation[28,30]. These findings suggest that alternate-day supplementation with low-dose ferrous bisglycinate capsules may lead to higher fractional iron uptake and fewer side effects compared to high-dose iron supplements taken daily or twice daily.

The effects of oral iron supplementation on iron deficiency-related symptoms in donors with low ferritin levels have currently only been studied in one non-blinded, non-randomized pilot study[31]. While many studies have shown beneficial effects of iron supplementation on iron store recovery time after whole blood donation, the optimal intake frequency and dosages are still unknown [13,32-34]. Similarly, the influence of dietary status and treatment adherence on post-donation iron store recovery using iron supplements has not been thoroughly assessed.

This study aims to determine the effect of iron supplementation on hemoglobin and ferritin levels, side effects, donor return, and iron deficiency-related symptoms in whole blood donors with low ferritin levels, thereby comparing varying intake frequencies and iron dosages.

Methods and analysis

Setting

Sanquin Blood Bank is a non-profit organization with a legal duty to collect, process, and provide blood products throughout the Netherlands[35]. Before every blood donation, the donor's eligibility to donate is assessed using a donor health questionnaire (DHQ) [36]. Donors should be in good health, aged between 18 and 79, and not at risk for any blood-borne infections. In accordance with European and international legislation, male and female whole blood donors with Hb ≤ 13.5 g/dL and Hb ≤ 12.5 g/dL (measured with the HemoCue 201, Angelholm, Sweden), respectively, are not eligible to donate for three months. Furthermore, routine ferritin measurements have been introduced since November 2017 for newly registered donors and at every 5th whole blood donation. Donors with ferritin levels ≥15 and ≤30 µg/L or <15 µg/L are deferred for 6 or 12 months, respectively.

Study population

Whole blood donors who have successfully donated before, donate at a participating blood bank location, are fluent in Dutch, and whose ferritin levels are measured during their next donation are invited by email to participate in the study. Donors are excluded from participation when regularly taking iron supplementation within the three months before enrolment. During their next donation, baseline measurements are taken, and a questionnaire is sent to donors who have indicated to be willing to participate. Inclusion into the trial is based on the baseline ferritin levels. Donors with baseline ferritin levels of ≤30 µg/L are eligible for the trial; for the other donors, the study ends after their next donation (i.e., baseline visit). All exclusion criteria are presented in figure 1.

Figure 1 Flowchart of the donor inclusion for the baseline questionnaire and the randomized controlled trial.

Study design

This is a 6-armed placebo-controlled double-blind, randomized controlled trial (RCT). Of the donors included at baseline, only those with ferritin levels ≤30 µg/L are eligible for participation in the trial and are randomly allocated to one of the six trial arms. The arms vary in (1) intake frequency; high frequency, or daily supplementation (HF), versus low frequency, or alternate day supplementation (LF), and (2) dosage; high dose (60mg elemental iron) iron supplements (HD), versus low dose (30mg elemental iron) iron supplements (LD), versus placebo (P). The inclusion of donors is continued until each trial arm consists of two hundred donors. We aim for an equal distribution of male and female participants (*Figure 2*).

Figure 2 Schematic diagram of the participant randomization. LF, low frequency supplementation (alternate day); HF, high frequency supplementation (daily); LD, low dose supplements (30mg elemental iron); HD, high dose supplements (60mg elemental iron); P, placebo supplements.

Study procedures

Shortly before the baseline and follow-up visits, donors are asked to complete an online questionnaire through Castor EDC [37] (Castor Electronic Data Capture, the Netherlands) and are provided with a hyperlink to the Wageningen University & Research page for an iron-specific food frequency questionnaire (FFQ).

At baseline, donors will visit one of the selected donation centers for a regular whole blood donation. When donors meet all eligibility criteria to donate, blood samples are taken from the blood donation sampling pouch. The collected blood samples are sent to the Sanquin National Screening Laboratory in Amsterdam for analysis. At least twice per week, the donor database containing donor ferritin levels is examined, and participating donors with ferritin levels ≤30 µg/L are selected. These donors are randomized and will receive iron or placebo supplements and additional study information through postal mail. Donors with ferritin levels >30µg/L are informed that their ferritin levels are sufficient and, therefore, they are not eligible

to participate in the RCT. During the follow-up visits, blood samples are collected through venipuncture.

Intervention and timeline

For this study, ferrous bisglycinate iron supplements are used with capsules containing 0, 30, or 60 mg of elemental iron. Participants are asked to adhere to the study product supplementation protocol as strictly as possible for 56 days after the baseline visit. The supplementation protocol instructs the participants to take the capsules at least three hours before and after eating products that interfere with iron uptake (e.g., dairy, coffee, tea, and soy). Therefore, participants are advised to take the capsules shortly before going to bed and asked to refrain from taking any additional iron supplements.

Follow-up visits will occur at 56, 122, and 182 days corresponding to the minimum donation interval for men, the minimum donation interval for women, and the minimum ferritin-guided deferral period in the Netherlands, respectively (*Figure 3)*. The participants are asked to return any unused capsules at the first follow-up visit to determine their treatment adherence.

Figure 3 Schematic diagram of the study timeline with t being the time since the baseline visit.

Sample size

Based on previous research performed by Kiss *et al.* and Waldvogel *et al.*, who used a similar randomized trial design in smaller groups of whole blood donors, sample size calculations were made for the primary outcome parameters, being ferritin and Hb, and the secondary outcome parameters, being adverse events, mental health, and physical health (Table 2)[18,38,39]. For the sample size calculation, we used the following formula:

$$n_1 = \frac{\left(z_{\left(\frac{1-\alpha}{2}\right)} + z_{(1-\beta)}\right)^2 * \sigma^2 * (r+1)}{v^2 * r}$$

Here, n_1 is the sample size of the intervention groups, r is the ratio between the control groups and the intervention groups, σ^2 is the variance of the continuous variable, v describes the expected difference between the continuous variables between the intervention and control groups (Table 2), $z_{\frac{1-\alpha}{2}}$ is the two-tailed alternative hypothesis with significance level α = 0.05 (1.96), $z_{(1-\beta)}$ is the probability of rejecting the null hypothesis when it is one minus probability of type II error (β) with a power of 0.90 (1.28).

It is expected that the chosen sample size is sufficient to reach statistical power for nearly all main outcome parameters (Table 2)[39]. For mental health, a lack of power is expected. However, indications of trends towards an effect might be observed, and data may be used in future meta-analyses to reach statistical significance. Furthermore, we expect to observe small differences in outcomes between low- and high-dose iron supplementation groups. To observe an expected difference (v) of 4 µg/l in ferritin and 2.5 g/l in hemoglobin between the iron supplementation groups, at least 126 and 151 donors should be included in each intervention group, respectively. Therefore, to reduce the risk of underpowering the study and considering possible dropout, we will include 200 participants per intervention group. Moreover, this will allow us to perform subgroup analysis based on sex, treatment adherence, and dietary intake.

	Iron supplementation	Placebo	Required sample size	Sufficiency
Hb	*13.4 (1.1) g/dL*	*12.0 (1.2) g/dL*	*19*	*Sufficient*
Ferritin	*28.0 (9.8) µg/L*	*12.9 (8.3) µg/L*	*14*	*Sufficient*
Adverse events*	*39.2%*	*15.5%*	*144*	*Sufficient*
Mental health**	*40.1 (4.8)*	*40.7 (4.8)*	*2016*	*Not sufficient*
Physical condition**	*54.8 (3.3)*	*52.4 (5.2)*	*60*	*Sufficient*

Table 1 Overview of the sample size calculations for Hb; ferritin; adverse events; mental health; physical health.

** Adverse events included gastrointestinal symptoms, dizziness, headache, acne, palpitations, and renal lithiasis.*
*** The outcome parameters mental health and physical condition were based on construct scores from the SF-12 questionnaire.*

Recruitment

Donors will first be recruited for the baseline questionnaire and blood sample measurements. The invitation is sent by email at least three weeks before the end of the donor's standard

donation interval or deferral period. Donors are provided with study information and an example of the informed consent form (appendix A). The information folder contains information about the study procedures and the rights of the participant. The invited donors are asked to respond to the invitation through email to indicate if they would like to participate in the study before signing the informed consent form. During the blood donation visit (i.e., baseline), donors who have agreed to participate are asked to sign the written informed consent form in a blood bank employee's presence. By signing the informed consent form, donors officially agree to participate and confirm that their personal information and material can be used for research purposes. Finally, the consent form is signed by the blood bank employee to affirm being the study representative, after which the donor can continue with the regular whole blood donation. Participation in this study is voluntary, and donors will not receive any compensation besides travel expenses as part of regular Sanquin Blood Bank policies.

We expect that fifty percent of the donors recruited for the baseline measurements have ferritin levels ≤30 µg/L and are eligible to participate in the trial[3]. Recruitment will continue until 1200 donors have been included in the trial. Data on demographics such as sex, age, donation history, and region are collected from the blood bank information system eProgesa (MAK systems, Paris, France). We aim to include all participants within one year and expect to finalize all follow-up visits at the end of 2022.

The trial is initiated at one blood collection center. Based on donor response- and inclusion rates, additional centers are added. The additional centers are added based on their capacity and ability to cope with the additional study-related workload, accessibility, the number of regularly donating donors, and the availability of direct transport to the National Screening Laboratory of Sanquin (NSS). Based on previous studies performed at Sanquin Research, we expect a response rate between 50% and 75%[40,41]. The expected response rate corresponds

with the inclusion of 1614 to 2421 eligible donors for the RCT per year, based on recruitment from two large Sanquin Blood Bank locations. In case of lower response rates, donors from other locations will also be included.

Blinding and randomization

Participant randomization is done on the individual level using a block randomization method. This procedure is realized through Castor EDC, with randomized block sizes set at 12 and 18. Furthermore, the randomization is stratified by age (18-49 years versus 50 years and older) and sex to account for menopausal effects in women, differences in ferritin levels between men and women, and the impact of aging on iron absorption[42-44]. Supplements and information are sent to the participants based on the number corresponding with the group they are allocated to after randomization. The responsible researcher is blinded for which group number corresponds with which study product. The 30mg iron, 60 mg iron, and placebo capsules are identical in terms of appearance and weight to guarantee the blinding of the participants and involved researchers. The participants will not be blinded for the varying intake frequency.

Data collection

Data are collected at baseline and during three follow-up visits. The baseline questionnaire consists of a combination of previously used or validated questionnaires. Participants are characterized by social-economic status, medical background, physical fitness, menstrual status, and smoking, as described in previous research[41]. To determine the effects of iron supplementation, we will use the *36-Item Short-Form Health Survey (SF-36)*; to determine baseline status and changes in general health[45], the *International Physical Activity Questionnaire Short Form (IPAQ)*; to assess changes in levels of physical activity[46], the Fatigue Assessment Scale; to assess changes in self-reported fatigue[47], donation intention-specific *Theory of Planned Behavior* questions; to determine if donors are more or less willing to donate as a consequence of iron supplementation[48], and *the Gastrointestinal Symptom Rating Scale*

and the *Bristol Stool Chart;* to assess the gastrointestinal side effects potentially caused by iron supplementation[49]. To determine any effects of iron supplementation on iron deficiency-related symptoms, we use the *Cambridge Hopkins Restless Legs Syndrome Questionnaire;* to assess the presence of and changes in restless legs, the *PICA questionnaire;* to assess a potential appetite for non-nutritive substances, and the *Cognitive Failure Questionnaire;* to determine changes in cognitive function[50-52].

Questionnaires that will also determine possible confounding and effect modification are the Treatment Adherence Questionnaire[53] to determine the participants' compliance with the intake protocols and an iron specific Food Frequency Questionnaire (FFQ) to determine their dietary iron, macro-, and micronutrient intake[41,54,55]. The FFQ allows us to assess whether or not iron supplements are more effective for donors who have low dietary (heme) iron intake and will only be completed at baseline and before the final follow-up visit. The follow-up visit questionnaires are similar to the baseline questionnaire, excluding the questions related to demographic characteristics and the addition of the adherence questionnaire for the first follow-up measurement. Furthermore, participants are asked to use the MedApp (MedApp Nederland B.V., Eindhoven, The Netherlands, https://medapp.nl) to assist with compliance with the supplementation protocol and to accurately assess treatment adherence. The MedApp will provide participants with daily or alternate daily notifications to remind them about their capsule intake and to indicate if the intake protocol was successfully followed.

At baseline and during follow-up, whole blood and serum samples are collected using 2ml, and 6ml coated EDTA (VACUETTE®, K3EDTA, Greiner Bio-one International GmbH, Austria) and 3.5ml and 5ml serum separating (VACUETTE®, Serum gel, Greiner Bio-one International GmbH, Austria) tubes, respectively. Ferritin measurements (Architect Ci8200, Abbott Laboratories, IL, USA), using serum samples, are performed routinely within 24 hours after the donation. The Architect Ci8200 is calibrated yearly for ferritin measurements by the manufacturer (Abbott Laboratories) and traceable to the the first WHO Human Liver Ferritin International Standard

(80/602). Furthermore, quality assurance assessments are performed daily by the laboratory staff, using low (20 µg/L), medium (150 µg/L), and high (400 µg/L) ferritin quality controls, provided by the manufacturer. When the daily quality assurance measurements do not meet the predefined acceptance criteria , the Architect Ci8200 will be recalibrated by the manufacturer (Abbott Laboratories). Quality management is in accordance with ISO 15189. Complete blood count measurements, including Hb and red blood cell parameters (Advia 2120, Siemens Medical Solutions Diagnostics, Breda, the Netherlands), are performed within 24 hours after the whole blood samples are taken. DNA is isolated using 400 µl buffy coat from EDTA- whole bloodsamples (QIAsymphony® DSP DNA Mini Kit, Qiagen GmbH, Hilden, Germany)[56] and stored at -20°C, for later use. Additional processed and aliquoted plasma and serum samples are collected from the EDTA- and serum separating tubes, respectively. The additionals samples will be stored at -80°C and used for potential post-study measurements of parameters which might affect the iron hemeostatis (e.g., inflammatory markers).

Statistical analysis

Descriptive statistics are presented for intervention and control groups and for men and women separately as means ± standard deviation for normally distributed data and median and interquartile range in case of a skewed distribution. For the analysis of the primary study parameters, the following multiple regression model is used if all assumptions for the model are fulfilled:

$$Effect = Intercept + ß1 * Dose_1 + ß2 * \mathrm{Dose}_2 + ß3 * \mathrm{Frequency} + ß4 * \mathrm{Dose}_1\mathrm{Frequency} + ß5 * Dose_2\mathrm{Frequency} + e$$

Here, *Intercept* represents the expected mean value when all explanatory variables have a value of 0, $Dose_1$ the low dose iron capsules (30mg), $Dose_2$ the high dose iron capsules (60mg), both dummy variables have 0mg as a reference, *frequency* the every other day versus daily intake, and *e* the error term or difference between observed and expected values. The assessed assumptions are the normality distributed random error term and the assumption of

the equality of variance. All the analyses will consist of two-sided tests, with a p-value <0.05 considered statistically significant. Statistical data analyses are performed with SPSS (IBM® SPSS® Statistics 23.0 or newer) and R (R Core Team, Windows)[57].

Potential effect modification by age, BMI, donation history, dietary intake (e.g., heme- and non-heme iron, dairy products, alcohol, tea, coffee, and total energy), and menstrual status are examined. These potential effect modifiers have been selected due to their role in iron hemoestasis[58,59]. We will test for effect modification by adding the variable of interest and an interaction term with the iron different iron dosages and intake frequency to the model. A variable is treated as an effect modifier when it has an interaction term with a p-value <0.10 for more than half of the associations. When effect modification is observed, stratified results will be reported in addition to the overall results. The following potential confounders are examined; age, sex, BMI, donation history, menstrual status, smoking, season, compliance with the study products, social-economic status, ethnicity, recent infections (including Sars-CoV-2), and baseline ferritin and Hb levels. A change of more than 10% in the regression coefficient is considered confounding, and variables are added to all the models.

Monitoring

Study monitoring is performed by the TAPAS Group, an independent monitoring bureau. The FORTE project has been labeled as a negligible risk study by the involved medical ethics committee based on a risk assessment. Study monitoring will consist of an initiation visit, two monitoring visits, and a close-out visit.

Patient and public involvement

Donors, as well as non-donors, are involved in the FORTE research project through focus group interviews. The focus group interviews will consist of interactive group discussions involving frequent donors; donors who have donated at least five times, new donors; donors who have signed up for donation but have not yet donated, and blood bank staff, including donor

physicians. During the focus group interviews, the participants are asked to discuss their perceptions, opinions, and attitude towards iron supplementation, current or potential alternative blood bank policies regarding iron management, and any potential effects of these aspects on their willingness to donate. Based on the outcomes of the focus group interviews, we will design a questionnaire to quantify the findings from the focus group interviews. The questionnaire is developed based on the recurrent constructs and topics observed during the group discussions and distributed amongst a larger group of donors and new donors. The results from the focus group interviews and the questionnaire are used to determine the optimal approach and potential areas of concern for implementing iron supplementation as a blood bank policy, if shown effective based on the trial's outcome.

ETHICS AND DISSEMINATION

Ethical considerations

This study is performed according to the Declaration of Helsinki and Good Clinical Practice guidelines. The Medical Research Ethics Committee (METC) of the Academic Medical Centre (AMC) Amsterdam has approved the study protocol (trial ID NL8590). All participants are asked to provide their written informed consent and are informed that they can discontinue participation at any time. Data collection is compliant with the General Data Protection Regulation (GDPR), and all data are pseudonymized to prevent the identification of study participants. To ensure the donors' safety, invasive actions such as venipuncture are performed by trained blood bank employees, following the routine blood donation protocols of Sanquin Blood Bank, the Netherlands, whenever applicable.

Dissemination

Study results are published in peer-reviewed journals after evaluation of scientific relevance and quality by the involved researchers. Furthermore, the results are presented at (inter)national conferences, shared with study participants, and communicated with donors and different Sanquin departments. Data that can lead to the identification of the participants will not be published.

SIGNIFICANCE AND OUTLOOK

This study's outcomes will provide evidence to lead the debate if and how iron supplementation should be implemented to support iron repletion for whole blood donors with low ferritin levels. Blood donation and adequate blood availability are of great importance to guarantee patients' health in need of blood transfusions. However, frequent blood donation increases the risk of iron deficiency in repeat whole blood donors. To ensure sufficient availability of blood and blood products and safeguard donor health, the donors' iron status should be monitored and managed appropriately. However, international uniformity amongst blood services regarding policies to address the donors' iron stores is lacking. Therefore, the FORTE study aims to provide new insights regarding the efficacy of iron supplementation for whole blood donors with low ferritin levels. We do this by comparing the effectiveness of high and low-dose iron capsules versus placebo for daily and alternate-day supplementation protocols in whole blood donors, thereby investigating laboratory and health-related outcomes. The thorough characterization of participating donors will add to an enhanced understanding of the effectiveness of iron supplements under various circumstances.

Author contributions

J.K., K.v.d.H., M.S. designed the study protocol. J.K. wrote the manuscript with input from K.v.d.H., F.Q., M.S., D.S., and V.N., H.Z. and H.W. were consulted for medical and ethical input.

Funding statement

Sanquin supported this research project: Product and Process Development Cellular Products Grant, project id: PPOC19-02/1-239.

Competing interests statement

The authors declare that they have no competing interests.

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Word count

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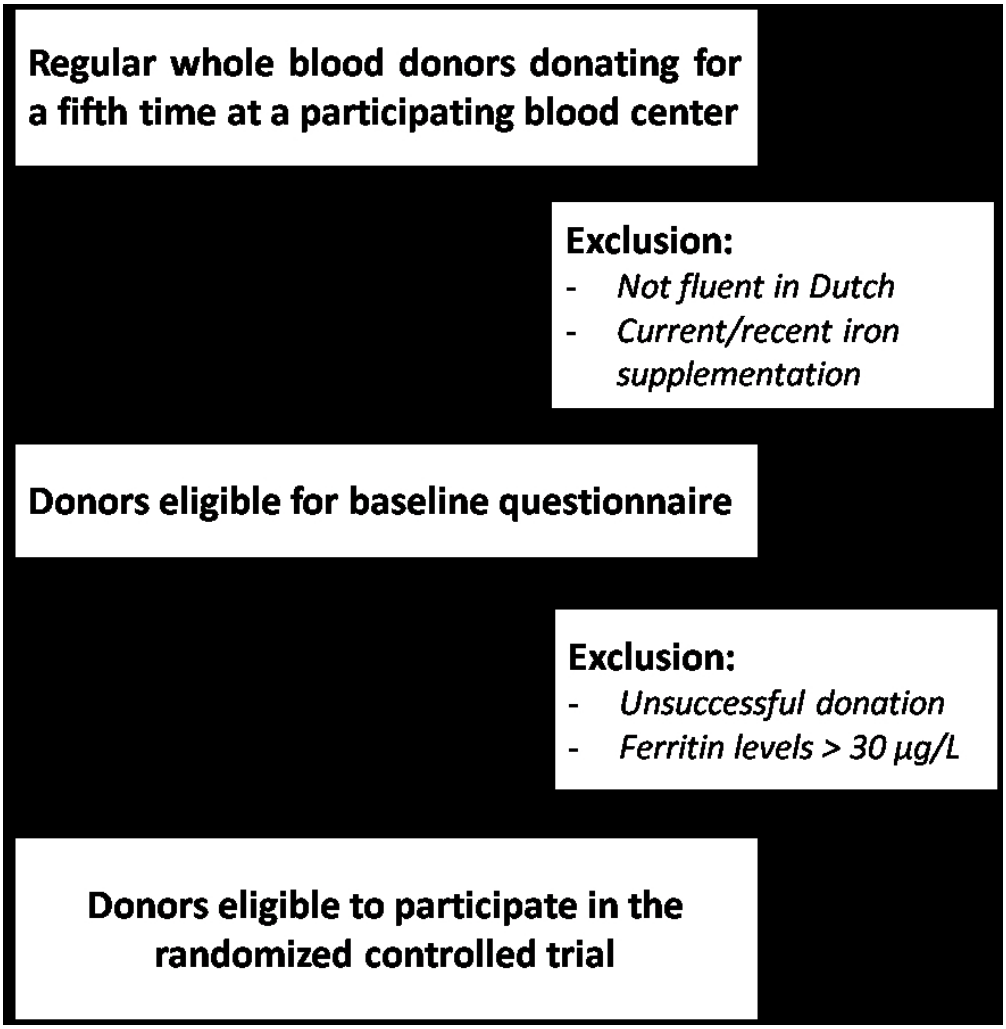
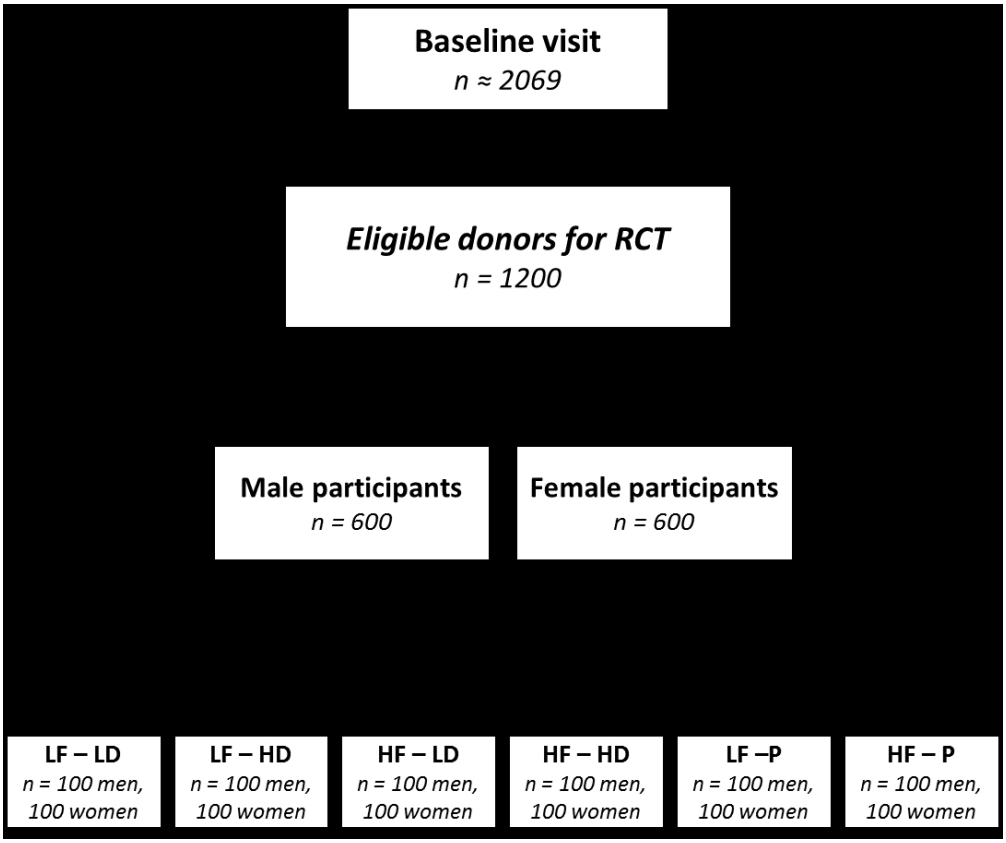


392x399mm (57 x 57 DPI)



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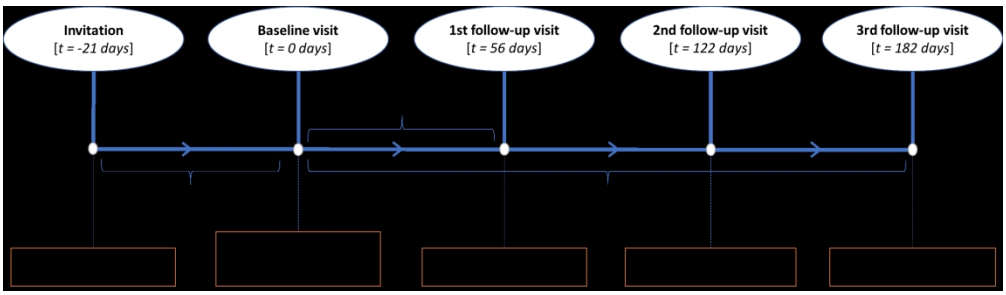

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Toestemmingsformulier FORTE

Onderzoek naar ijzersuppletie bij bloeddonors met een laag ferritine

- Ik heb de informatiebrief gelezen. Ook kon ik vragen stellen. Mijn vragen zijn voldoende beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.

- Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen of te stoppen met het onderzoek. Daarvoor hoef ik geen reden te geven.

- Ik geef toestemming dat er informatie kan worden opgevraagd bij een medisch centrum, bij mijn huisarts, of het Centraal Bureau voor de Statistiek (CBS) over mijn gezondheid, een ziekenhuisopname, of overlijden tijdens of na afloop van het onderzoek.

- Ik geef toestemming voor het koppelen van mijn donatiegegevens aan de onderzoeksgegevens.

- Ik geef toestemming dat mijn gegevens en lichaamsmateriaal worden verzameld, gebruikt, en bewaard voor de beantwoording van de onderzoeksvraag in dit onderzoek.

- Ik weet dat voor de controle van het onderzoek sommige mensen toegang tot al mijn gegevens kunnen krijgen. Deze mensen staan vermeld in deze informatiebrief. Ik geef toestemming voor inzage door deze personen.

- Ik geef toestemming dat Sanquin mijn gegevens en lichaamsmateriaal mag bewaren tot 15 jaar na het onderzoek.

- Ik geef toestemming dat mijn gecodeerde gegevens en lichaamsmaterialen mogen worden gedeeld met andere binnen- en buitenlandse laboratoria en onderzoekers voor doelen zoals beschreven in de informatiebrief.

- Ik geef □ wel
  □ geen
  toestemming om DNA uit mijn bloed te halen en dit te bewaren en te gebruiken voor dit onderzoek.

- Ik geef □ wel
  □ geen
  toestemming om mij opnieuw te benaderen voor een mogelijk vervolgonderzoek.

- Ik geef □ wel
  □ geen
  toestemming voor nog 15 jaar extra opslag, na de 15 jaar waarin het lichaamsmateriaal, DNA en de onderzoeksgegevens reeds in het kader van deze studie worden bewaard, in totaal dus 30 jaar.

- Ik wil deelnemen aan het onderzoek.

Naam proefpersoon:……………………………..

Donornummer: …………………………………...

Handtekening:……………………………………………………. Datum: __ / __ / __

Ik verklaar dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek.

Als er tijdens het onderzoek informatie bekend wordt die de toestemming van de proefpersoon zou kunnen beïnvloeden, dan breng ik hem/haar daarvan tijdig op de hoogte.

Naam onderzoeker (of diens vertegenwoordiger): ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,……

Handtekening:…………………………………………………….. Datum: __ / __ / __

---.

EIN-sticker:

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	x
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	19
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	19
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	x
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	x

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	17
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9-10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	12
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	x
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14-15
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	x
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	15
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	x
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	17

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	17
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	17
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	19
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	x
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	17
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	x
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	x

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Ferritin-guided iron supplementation in whole blood donors: Optimal dosage, donor Response, return, and Efficacy (FORTE) – a randomized controlled trial protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-056316.R1
Article Type:	Protocol
Date Submitted by the Author:	14-Jan-2022
Complete List of Authors:	Karregat, Jan; Sanquin Research, Department of Donor Medicine Research Sweegers, Maike G; Sanquin Research, Department of Donor Medicine Research Quee, Franke A; Sanquin Research, Department of Donor Medicine Research Weekamp, Henriëtte H; Sanquin Blood Supply Foundation, Medical Donor Affairs Swinkels, Dorine W; Radboud University Nijmegen, Department of Laboratory Medicine; Sanquin Blood Supply Foundation, Center for Iron Disorders Sanquin Novotny, Věra M J; Sanquin Blood Supply Foundation, Department of Transfusion Medicine Zaaijer , Hans L; Sanquin Research, Department of Donor Medicine Research; Amsterdam UMC Location AMC, Department of Clinical Virology van den Hurk, Katja; Sanquin Research, Department of Donor Medicine Research
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Epidemiology, Public health
Keywords:	Blood bank & transfusion medicine < HAEMATOLOGY, Anaemia < HAEMATOLOGY, Clinical trials < THERAPEUTICS

Ferritin-guided iron supplementation in whole blood donors: Optimal dosage, donor Response, return, and Efficacy (FORTE) – a randomized controlled trial protocol

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Protocol version 2, 13-01-2022

Word count: 3770

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Abstract

Background: Frequent whole blood donors have an increased risk of developing iron deficiency. Iron deficiency can have detrimental health effects when left untreated. Donation intervals are commonly too short to replenish iron stores, and extending these reduces donor availability. Oral iron supplementation is known to shorten iron store recovery time but may also induce gastrointestinal complaints. We aim to optimize the effectiveness of iron supplements while minimizing the risks of side effects. Therefore, we will evaluate the impact of different iron supplementation protocols in terms of dosage and frequency on ferritin and hemoglobin levels, gastrointestinal side-effects, iron deficiency-related symptoms, and donor return compared to placebo supplementation.

Methods: Twelve hundred whole blood donors with ferritin levels ≤30 µg/L are included into a double-blind, randomized controlled trial. Participants are randomly allocated to one of six arms, administering capsules containing 0, 30, or 60 mg of iron, either on alternate days or daily for 56 days. At baseline and 56, 122, and 182 days of follow-up, ferritin and hemoglobin levels are measured, and compliance, donor return, dietary iron intake, gastrointestinal, iron deficiency-related symptoms, and general health are assessed by questionnaire.

Ethics and dissemination: This study will provide a comprehensive overview of the effects of different frequencies and dosages of administration of iron supplements on iron status and health effects, thereby considering individual differences in treatment adherence and lifestyle. The outcome will provide scientific evidence to guide the debate if and how oral iron supplements may support the recovery of whole blood donors with low ferritin levels.

Trial registration: The Dutch trial registry NL8590.

Strengths and limitations of this study

- This is a large (n = 1200), double-blind, randomized controlled trial to determine the optimal iron supplementation protocol in terms of both intake frequency and dosage.
- Outcome variables include ferritin and hemoglobin levels, complete blood counts, iron deficiency-related symptoms, and gastrointestinal side effects.
- Participants are thoroughly characterized at baseline regarding social-economic status, medical background, physical fitness, dietary intake, and smoking status.
- A limitation is the limited number of follow-up visits; however, these time points are the most relevant, and temporal patterns are modeled based on earlier studies.

Introduction

Due to the hemoglobin (Hb)-bound iron loss during donation, regular whole blood donors are prone to developing iron deficiency, often in the absence of anemia[1,2]. Iron deficiency without anemia is characterized by reduced serum ferritin levels. It is highly prevalent amongst frequent whole blood donors, with 15.0% of the female and 9.4% of male Dutch donors having ferritin levels <15 µg/L [3]. Reduced ferritin levels increase the risk of developing anemia and are associated with iron deficiency-related symptoms, including fatigue, reduced exercise endurance, restless legs, PICA (appetite for non-nutritious substances), and reduced neurocognitive functioning[4-8].

At most international blood banks, including the Netherlands, Hb levels are measured before donation to safeguard donor health and blood product quality[9]. However, Hb levels do not reflect the amount of stored iron, which is significantly impacted by whole blood donations[10]. Therefore, ferritin measurements have become more common amongst blood banks to assess the donor's iron storage. Sanquin incorporated ferritin-guided donation intervals, deferring donors for 6 or 12 months when ferritin levels are ≥15 and ≤30 µg/L or <15 µg/L, respectively. These cut-off values are based on WHO standards, as described previously[3]. However, donor deferral has been shown to demoralize donors and reduce donor return rates [3,11,12].

Several studies have shown that oral iron supplementation reduces the post-donation recovery time of ferritin and Hb levels to pre-donation levels[13,14]. While donors in the countries like the United States, Finland, and Denmark are already advised about iron supplementation for iron storage recovery after donation, other blood services are hesitant, often due to ethical concerns[15-17]. Kiss *et al.* showed in a randomized controlled trial that post-donation iron supplementation led to full recovery of ferritin levels within 56 days, whereas the non-iron supplementing group did not reach full recovery after 160 days [18].

In several clinical trials, the iron supplement ferrous bisglycinate has been shown to result in a relatively high fractional iron uptake. This has been attributed to its iron-bound chelates that prevent the iron from binding to other dietary compounds (e.g., tannins, catechols, and phytates) that inhibit iron absorption[19-22]. Furthermore, supplementation with ferrous bisglycinate has been shown to lower the risk of gastrointestinal discomfort compared to other iron formulations, possibly due to the increased uptake by intestinal mucosal cells, leading to less iron entering the colon[19,23,24]. Therefore, ferrous bisglycinate is already used by the Danish blood bank for donors who suffer from gastrointestinal side-effects[25,26].

Iron absorption in the duodenum and its entry into the plasma compartment is regulated by the peptide hormone hepcidin[27]. It has been shown that iron supplementation leads to an increased hepatic production of hepcidin in iron-depleted women, causing a reduced intestinal iron uptake up to 24 hours post-supplementation[28,29]. While the dosages of elemental iron used in previous studies range from 19 to 240 mg/day, lower dosed iron supplements are shown effective in recovering the iron stores post-donation[28,30]. These findings suggest that alternate-day supplementation with low-dose ferrous bisglycinate capsules may lead to higher fractional iron uptake and fewer side effects compared to high-dose iron supplements taken daily or twice daily.

The effects of oral iron supplementation on iron deficiency-related symptoms in donors with low ferritin levels have currently only been studied in one non-blinded, non-randomized pilot study[31]. While many studies have shown beneficial effects of iron supplementation on iron store recovery time after whole blood donation, the optimal intake frequency and dosages are still unknown [13,32-34]. Similarly, the influence of dietary status and treatment adherence on post-donation iron store recovery using iron supplements has not been thoroughly assessed.

This study aims to determine the effect of iron supplementation on hemoglobin and ferritin levels, side effects, donor return, and iron deficiency-related symptoms in whole blood donors with low ferritin levels, thereby comparing varying intake frequencies and iron dosages.

Methods and analysis

Setting

Sanquin Blood Bank is a non-profit organization with a legal duty to collect, process, and provide blood products throughout the Netherlands[35]. Before every blood donation, the donor's eligibility to donate is assessed using a donor health questionnaire (DHQ) [36]. Donors should be in good health, aged between 18 and 79, and not at risk for any blood-borne infections. In accordance with European and international legislation, male and female whole blood donors with Hb ≤ 13.5 g/dL and Hb ≤ 12.5 g/dL (measured with the HemoCue 201, Angelholm, Sweden), respectively, are not eligible to donate for three months. Furthermore, routine ferritin measurements have been introduced since November 2017 for newly registered donors and at every 5th whole blood donation. Donors with ferritin levels ≥15 and ≤30 µg/L or <15 µg/L are deferred for 6 or 12 months, respectively.

Study population

Whole blood donors who have successfully donated before, donate at a participating blood bank location, are fluent in Dutch, and whose ferritin levels are measured during their next donation are invited by email to participate in the study. Donors are excluded from participation when regularly taking iron supplementation within the three months before enrolment. During their next donation, baseline measurements are taken, and a questionnaire is sent to donors who have indicated to be willing to participate. Inclusion into the trial is based on the baseline ferritin levels. Donors with baseline ferritin levels of ≤30 µg/L are eligible for the trial; for the other donors, the study ends after their next donation (i.e., baseline visit). All exclusion criteria are presented in figure 1.

Study design

This is a 6-armed placebo-controlled double-blind, randomized controlled trial (RCT). Of the donors included at baseline, only those with ferritin levels ≤30 µg/L are eligible for participation in the trial and are randomly allocated to one of the six trial arms. The arms vary in (1) intake frequency; high frequency, or daily supplementation (HF), versus low frequency, or alternate day supplementation (LF), and (2) dosage; high dose (60mg elemental iron) iron supplements (HD), versus low dose (30mg elemental iron) iron supplements (LD), versus placebo (P). The inclusion of donors is continued until each trial arm consists of two hundred donors. We aim for an equal distribution of male and female participants (*Figure 2*).

Study procedures

Shortly before the baseline and follow-up visits, donors are asked to complete an online questionnaire through Castor EDC [37] (Castor Electronic Data Capture, the Netherlands) and are provided with a hyperlink to the Wageningen University & Research page for an iron-specific food frequency questionnaire (FFQ).

At baseline, donors will visit one of the selected donation centers for a regular whole blood donation. When donors meet all eligibility criteria to donate, blood samples are taken from the blood donation sampling pouch. The collected blood samples are sent to the Sanquin National Screening Laboratory in Amsterdam for analysis. At least twice per week, the donor database containing donor ferritin levels is examined, and participating donors with ferritin levels ≤30 µg/L are selected. These donors are randomized and will receive iron or placebo supplements and additional study information through postal mail. Donors with ferritin levels >30µg/L are informed that their ferritin levels are sufficient and, therefore, they are not eligible

to participate in the RCT. During the follow-up visits, blood samples are collected through venipuncture.

Intervention and timeline

For this study, ferrous bisglycinate iron supplements are used with capsules containing 0, 30, or 60 mg of elemental iron. Participants are asked to adhere to the study product supplementation protocol as strictly as possible for 56 days after the baseline visit. The supplementation protocol instructs the participants to take the capsules at least three hours before and after eating products or taking medicine that interfere with iron uptake (e.g., dairy, coffee, tea, soy, antacids, and anti-biotics). Therefore, participants are advised to take the capsules shortly before going to bed and asked to refrain from taking any additional iron supplements.

Follow-up visits will occur at 56, 122, and 182 days corresponding to the minimum donation interval for men, the minimum donation interval for women, and the minimum ferritin-guided deferral period in the Netherlands, respectively (*Figure 3)*. The participants are asked to return any unused capsules at the first follow-up visit to determine their treatment adherence.

Sample size

Based on previous research performed by Kiss *et al.* and Waldvogel *et al.*, who used a similar randomized trial design in smaller groups of whole blood donors, sample size calculations were made for the primary outcome parameters, being ferritin and Hb, and the secondary outcome parameters, being adverse events, mental health, and physical health (Table 1)[18,38,39]. For the sample size calculation, we used the following formula:

$$n_1 = \frac{\left(z_{\left(\frac{1-\alpha}{2}\right)} + z_{(1-\beta)}\right)^2 * \sigma^2 * (r+1)}{v^2 * r}$$

Here, n_1 is the sample size of the intervention groups, r is the ratio between the control groups and the intervention groups, σ^2 is the variance of the continuous variable, v describes the expected difference between the continuous variables between the intervention and control groups (Table 1), $z_{\frac{1-\alpha}{2}}$ is the two-tailed alternative hypothesis with significance level α = 0.05 (1.96), $z_{(1-\beta)}$ is the probability of rejecting the null hypothesis when it is one minus probability of type II error (β) with a power of 0.90 (1.28).

It is expected that the chosen sample size is sufficient to reach statistical power for nearly all main outcome parameters (Table 1)[39]. For mental health, a lack of power is expected. However, indications of trends towards an effect might be observed, and data may be used in future meta-analyses to reach statistical significance. Furthermore, we expect to observe small differences in outcomes between low- and high-dose iron supplementation groups. To observe an expected difference (v) of 4 µg/l in ferritin and 2.5 g/l in hemoglobin between the iron supplementation groups, at least 126 and 151 donors should be included in each intervention group, respectively. Therefore, to reduce the risk of underpowering the study and considering possible dropout, we will include 200 participants per intervention group. Moreover, this will allow us to perform subgroup analysis based on sex, treatment adherence, and dietary intake.

	Iron supplementation	Placebo	Required sample size	Sufficiency
Hb	*13.4 (1.1) g/dL*	*12.0 (1.2) g/dL*	*19*	*Sufficient*
Ferritin	*28.0 (9.8) µg/L*	*12.9 (8.3) µg/L*	*14*	*Sufficient*
Adverse events*	*39.2%*	*15.5%*	*144*	*Sufficient*
Mental health**	*40.1 (4.8)*	*40.7 (4.8)*	*2016*	*Not sufficient*
Physical condition**	*54.8 (3.3)*	*52.4 (5.2)*	*60*	*Sufficient*

Table 1 Overview of the sample size calculations for Hb; ferritin; adverse events; mental health; physical health.

** Adverse events included gastrointestinal symptoms, dizziness, headache, acne, palpitations, and renal lithiasis.*
*** The outcome parameters mental health and physical condition were based on construct scores from the SF-12 questionnaire.*

Recruitment

Donors will first be recruited for the baseline questionnaire and blood sample measurements. The invitation is sent by email at least three weeks before the end of the donor's standard

donation interval or deferral period. Donors are provided with study information and an example of the informed consent form (appendix A). The information folder contains information about the study procedures and the rights of the participant. The invited donors are asked to respond to the invitation through email to indicate if they would like to participate in the study before signing the informed consent form. During the blood donation visit (i.e., baseline), donors who have agreed to participate are asked to sign the written informed consent form in a blood bank employee's presence. By signing the informed consent form, donors officially agree to participate and confirm that their personal information and material can be used for research purposes. Finally, the consent form is signed by the blood bank employee to affirm being the study representative, after which the donor can continue with the regular whole blood donation. Participation in this study is voluntary, and donors will not receive any compensation besides travel expenses as part of regular Sanquin Blood Bank policies.

We expect that fifty percent of the donors recruited for the baseline measurements have ferritin levels ≤30 µg/L and are eligible to participate in the trial[3]. Recruitment will continue until 1200 donors have been included in the trial. Data on demographics such as sex, age, donation history, and region are collected from the blood bank information system eProgesa (MAK systems, Paris, France). Recruitment will start in July 2022. We aim to include all participants within one year and expect to finalize all follow-up visits at the end of 2022.

The trial is initiated at one blood collection center. Based on donor response- and inclusion rates, additional centers are added. The additional centers are added based on their capacity and ability to cope with the additional study-related workload, accessibility, the number of regularly donating donors, and the availability of direct transport to the National Screening Laboratory of Sanquin (NSS). Based on previous studies performed at Sanquin Research, we expect a response rate between 50% and 75%[40,41]. The expected response rate corresponds

with the inclusion of 1614 to 2421 eligible donors for the RCT per year, based on recruitment from two large Sanquin Blood Bank locations. In case of lower response rates, donors from other locations will also be included.

Blinding and randomization

Participant randomization is done on the individual level using a block randomization method. This procedure is realized through Castor EDC, with randomized block sizes set at 12 and 18. Furthermore, the randomization is stratified by age (18-49 years versus 50 years and older) and sex to account for menopausal effects in women, differences in ferritin levels between men and women, and the impact of aging on iron absorption[42-44]. Supplements and information are sent to the participants based on the number corresponding with the group they are allocated to after randomization. The responsible researcher is blinded for which group number corresponds with which study product. The 30mg iron, 60 mg iron, and placebo capsules are identical in terms of appearance and weight to guarantee the blinding of the participants and involved researchers. The participants will not be blinded for the varying intake frequency.

Data collection

Data are collected at baseline and during three follow-up visits. The baseline questionnaire consists of a combination of previously used or validated questionnaires. Participants are characterized by social-economic status, medical background, physical fitness, menstrual status, and smoking, as described in previous research[41]. To determine the effects of iron supplementation, we will use the *36-Item Short-Form Health Survey (SF-36)*; to determine baseline status and changes in general health[45], the *International Physical Activity Questionnaire Short Form (IPAQ)*; to assess changes in levels of physical activity[46], the Fatigue Assessment Scale; to assess changes in self-reported fatigue[47], donation intention-specific *Theory of Planned Behavior* questions; to determine if donors are more or less willing to donate as a consequence of iron supplementation[48], and *the Gastrointestinal Symptom Rating Scale*

and the *Bristol Stool Chart;* to assess the gastrointestinal side effects potentially caused by iron supplementation[49]. To determine any effects of iron supplementation on iron deficiency-related symptoms, we use the *Cambridge Hopkins Restless Legs Syndrome Questionnaire;* to assess the presence of and changes in restless legs, the *PICA questionnaire;* to assess a potential appetite for non-nutritive substances, and the *Cognitive Failure Questionnaire;* to determine changes in cognitive function[50-52].

Questionnaires that will also determine possible confounding and effect modification are the Treatment Adherence Questionnaire[53] to determine the participants' compliance with the intake protocols and an iron specific Food Frequency Questionnaire (FFQ) to determine their dietary iron, macro-, and micronutrient intake[41,54,55]. The FFQ allows us to assess whether or not iron supplements are more effective for donors who have low dietary (heme) iron intake and will only be completed at baseline and before the final follow-up visit. The follow-up visit questionnaires are similar to the baseline questionnaire, excluding the questions related to demographic characteristics and the addition of the adherence questionnaire for the first follow-up measurement. Furthermore, participants are asked to use the MedApp (MedApp Nederland B.V., Eindhoven, The Netherlands, https://medapp.nl) to assist with compliance with the supplementation protocol and to accurately assess treatment adherence. The MedApp will provide participants with daily or alternate daily notifications to remind them about their capsule intake and to indicate if the intake protocol was successfully followed.

At baseline and during follow-up, whole blood and serum samples are collected using 2ml, and 6ml coated EDTA (VACUETTE®, K3EDTA, Greiner Bio-one International GmbH, Austria) and 3.5ml and 5ml serum separating (VACUETTE®, Serum gel, Greiner Bio-one International GmbH, Austria) tubes, respectively. Ferritin measurements (Architect Ci8200, Abbott Laboratories, IL, USA), using serum samples, are performed routinely within 24 hours after the donation. The Architect Ci8200 is calibrated yearly for ferritin measurements by the manufacturer (Abbott Laboratories) and traceable to the the first WHO Human Liver Ferritin International Standard

(80/602). Furthermore, quality assurance assessments are performed daily by the laboratory staff, using low (20 µg/L), medium (150 µg/L), and high (400 µg/L) ferritin quality controls, provided by the manufacturer. When the daily quality assurance measurements do not meet the predefined acceptance criteria , the Architect Ci8200 will be recalibrated by the manufacturer (Abbott Laboratories). Quality management is in accordance with ISO 15189. Complete blood count measurements, including Hb and red blood cell parameters (Advia 2120, Siemens Medical Solutions Diagnostics, Breda, the Netherlands), are performed within 24 hours after the whole blood samples are taken. DNA is isolated using 400 µl buffy coat from EDTA- whole bloodsamples (QIAsymphony® DSP DNA Mini Kit, Qiagen GmbH, Hilden, Germany)[56] and stored at -20°C, for later use. Additional processed and aliquoted plasma and serum samples are collected from the EDTA- and serum separating tubes, respectively. The additionals samples will be stored at -80°C and used for potential post-study measurements of parameters which might affect the iron hemeostatis (e.g., inflammatory markers).

Statistical analysis

Descriptive statistics are presented for intervention and control groups and for men and women separately as means ± standard deviation for normally distributed data and median and interquartile range in case of a skewed distribution. For the analysis of the primary study parameters, the following multiple regression model is used if all assumptions for the model are fulfilled:

$$Effect = Intercept + ß1 * Dose_1 + ß2 * \mathrm{Dose}_2 + ß3 * \mathrm{Frequency} + ß4 * \mathrm{Dose}_1\mathrm{Frequency} + ß5 * Dose_2\mathrm{Frequency} + e$$

Here, *Intercept* represents the expected mean value when all explanatory variables have a value of 0, $Dose_1$ the low dose iron capsules (30mg), $Dose_2$ the high dose iron capsules (60mg), both dummy variables have 0mg as a reference, *frequency* the every other day versus daily intake, and *e* the error term or difference between observed and expected values. The assessed assumptions are the normality distributed random error term and the assumption of

the equality of variance. All the analyses will consist of two-sided tests, with a p-value <0.05 considered statistically significant. Statistical data analyses are performed with SPSS (IBM® SPSS® Statistics 23.0 or newer) and R (R Core Team, Windows)[57].

Potential effect modification by age, BMI, donation history, dietary intake (e.g., heme- and non-heme iron, dairy products, alcohol, tea, coffee, and total energy), and menstrual status are examined. These potential effect modifiers have been selected due to their role in iron hemoestasis[58,59]. We will test for effect modification by adding the variable of interest and an interaction term with the iron different iron dosages and intake frequency to the model. A variable is treated as an effect modifier when it has an interaction term with a p-value <0.10 for more than half of the associations. When effect modification is observed, stratified results will be reported in addition to the overall results. The following potential confounders are examined; age, sex, BMI, donation history, menstrual status, smoking, season, compliance with the study products, social-economic status, ethnicity, recent infections (including Sars-CoV-2), and baseline ferritin and Hb levels. A change of more than 10% in the regression coefficient is considered confounding, and variables are added to all the models.

Monitoring

Study monitoring is performed by the TAPAS Group, an independent monitoring bureau. The FORTE project has been labeled as a negligible risk study by the involved medical ethics committee based on a risk assessment. Study monitoring will consist of an initiation visit, two monitoring visits, and a close-out visit.

Patient and public involvement

Donors, as well as non-donors, are involved in the FORTE research project through focus group interviews. The focus group interviews will consist of interactive group discussions involving frequent donors; donors who have donated at least five times, new donors; donors who have signed up for donation but have not yet donated, and blood bank staff, including donor

physicians. During the focus group interviews, the participants are asked to discuss their perceptions, opinions, and attitude towards iron supplementation, current or potential alternative blood bank policies regarding iron management, and any potential effects of these aspects on their willingness to donate. Based on the outcomes of the focus group interviews, we will design a questionnaire to quantify the findings from the focus group interviews. The questionnaire is developed based on the recurrent constructs and topics observed during the group discussions and distributed amongst a larger group of donors and new donors. The results from the focus group interviews and the questionnaire are used to determine the optimal approach and potential areas of concern for implementing iron supplementation as a blood bank policy, if shown effective based on the trial's outcome.

ETHICS AND DISSEMINATION

Ethical considerations

This study is performed according to the Declaration of Helsinki and Good Clinical Practice guidelines. The Medical Research Ethics Committee (METC) of the Academic Medical Centre (AMC) Amsterdam has approved the study protocol (trial ID NL8590). All participants are asked to provide their written informed consent and are informed that they can discontinue participation at any time. Data collection is compliant with the General Data Protection Regulation (GDPR), and all data are pseudonymized to prevent the identification of study participants. To ensure the donors' safety, invasive actions such as venipuncture are performed by trained blood bank employees, following the routine blood donation protocols of Sanquin Blood Bank, the Netherlands, whenever applicable.

Dissemination

Study results are published in peer-reviewed journals after evaluation of scientific relevance and quality by the involved researchers. Furthermore, the results are presented at (inter)national conferences, shared with study participants, and communicated with donors and different Sanquin departments. Data that can lead to the identification of the participants will not be published.

SIGNIFICANCE AND OUTLOOK

This study's outcomes will provide evidence to lead the debate if and how iron supplementation should be implemented to support iron repletion for whole blood donors with low ferritin levels. Blood donation and adequate blood availability are of great importance to guarantee patients' health in need of blood transfusions. However, frequent blood donation increases the risk of iron deficiency in repeat whole blood donors. To ensure sufficient availability of blood and blood products and safeguard donor health, the donors' iron status should be monitored and managed appropriately. However, international uniformity amongst blood services regarding policies to address the donors' iron stores is lacking. Therefore, the FORTE study aims to provide new insights regarding the efficacy of iron supplementation for whole blood donors with low ferritin levels. We do this by comparing the effectiveness of high and low-dose iron capsules versus placebo for daily and alternate-day supplementation protocols in whole blood donors, thereby investigating laboratory and health-related outcomes. The thorough characterization of participating donors will add to an enhanced understanding of the effectiveness of iron supplements under various circumstances.

Author contributions

J.K., K.v.d.H., M.S. designed the study protocol. J.K. wrote the manuscript with input from K.v.d.H., F.Q., M.S., D.S.. V.N., H.Z. and H.W. were consulted for medical and ethical input.

Statement of Ethics Approval

This study involves human participants and was approved by the Medical Research Ethics Committee (METC) of the Academic Medical Centre (AMC) Amsterdam (reference number: 2020_206); trial ID NL8590. All participants are asked to provide their written informed consent and are informed that they can discontinue participation at any time.

Funding statement

Sanquin supported this research project: Product and Process Development Cellular Products Grant, project id: PPOC19-02/1-239.

Competing interests statement

The authors declare that they have no competing interests.

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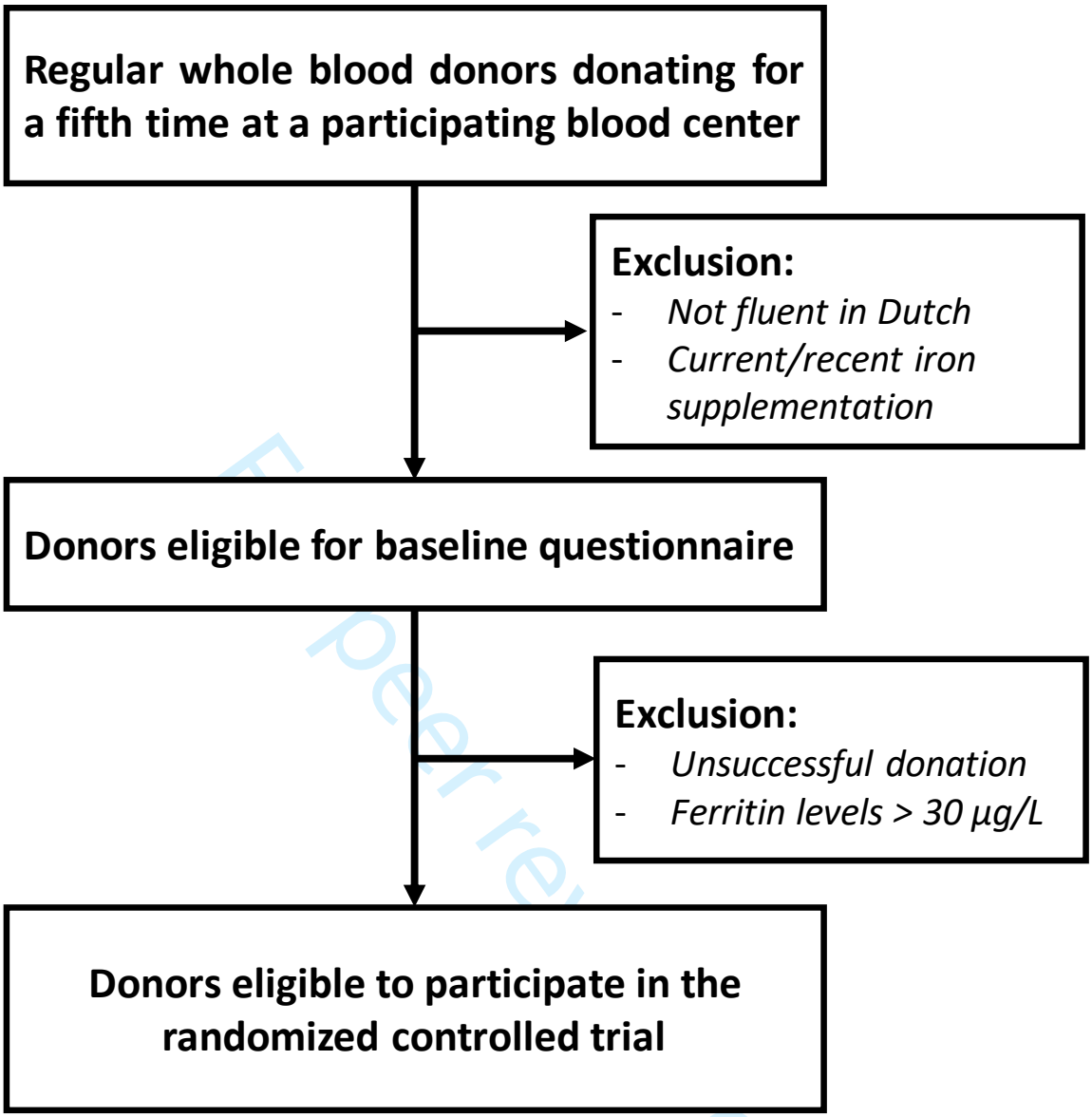
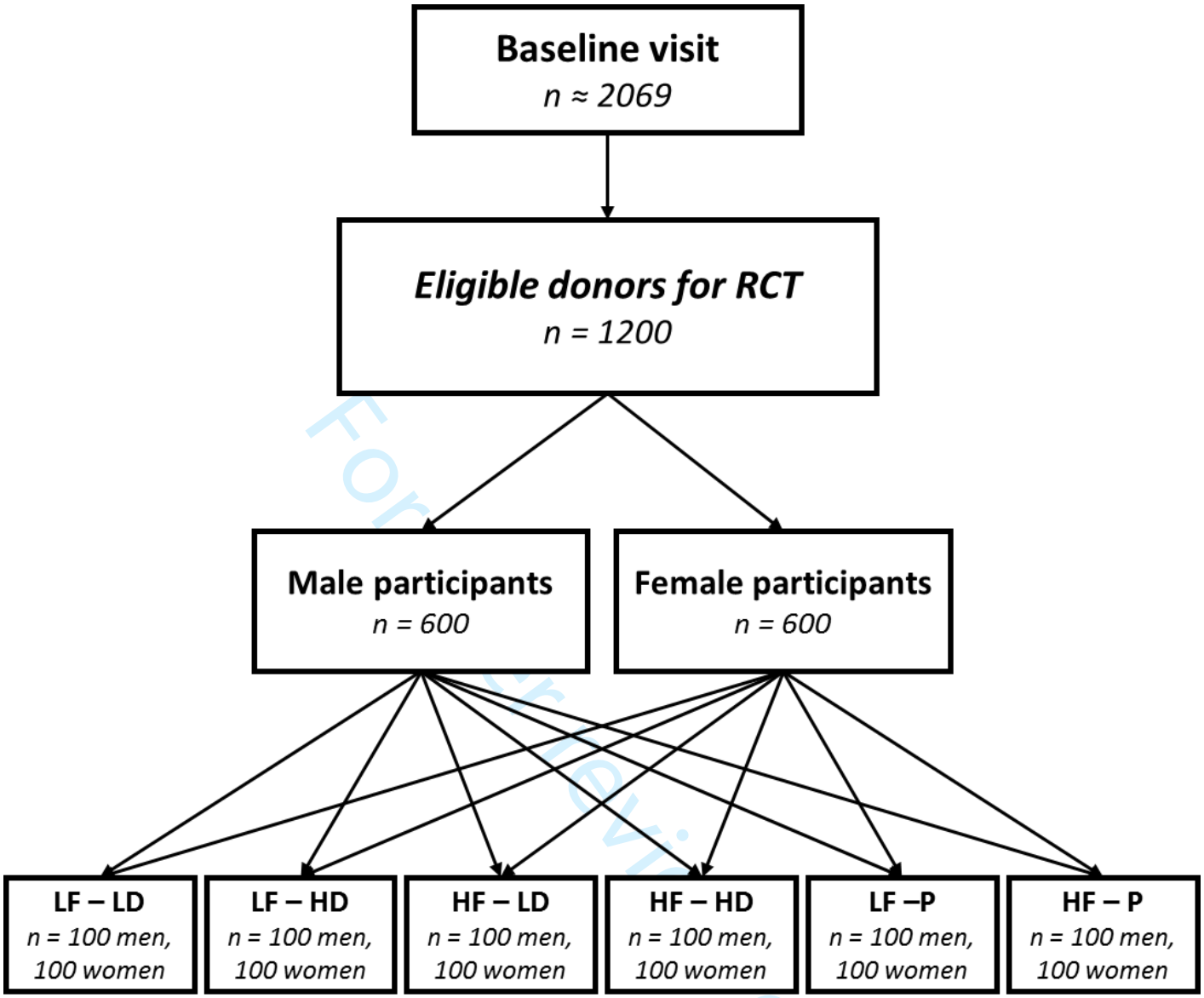
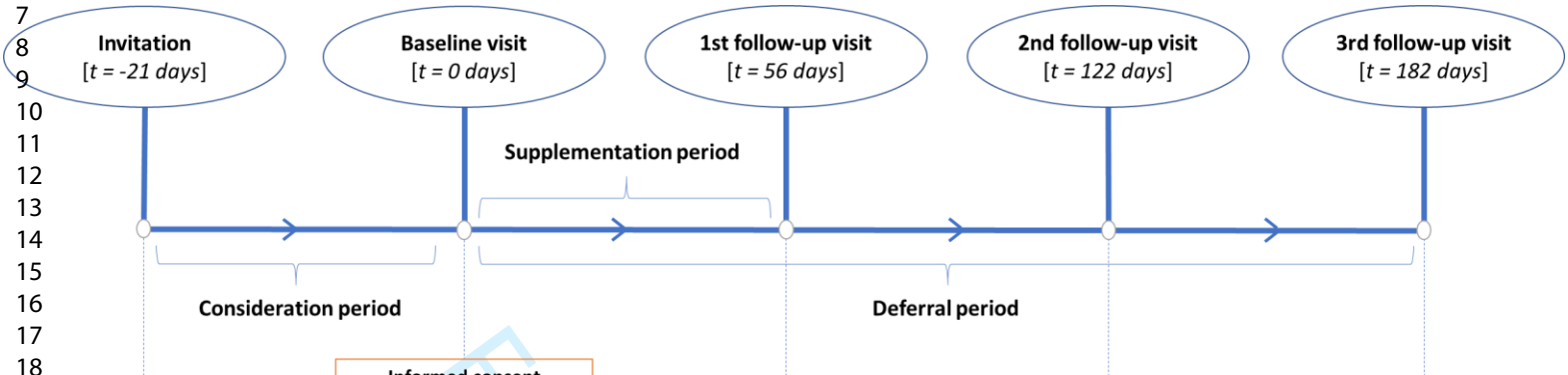
Figure 1 Flowchart of the donor inclusion for the baseline questionnaire and the randomized controlled trial.

Figure 3 Schematic diagram of the participant randomization. LF, low frequency supplementation (alternate day); HF, high frequency supplementation (daily); LD, low dose supplements (30mg elemental iron); HD, high dose supplements (60mg elemental iron); P, placebo supplements.

Figure 2 Schematic diagram of the study timeline with t being the time since the baseline visit.







Informed consent form FORTE

Research regarding iron supplementation for blood donors with low ferritin levels

- I have read the information letter. I was also able to ask questions. My questions have been answered adequately. I have had enough time to consider if I would like to participate.
- I understand that participation is voluntarily. I also know that I can decide to quit participating at any moment. For this I don't need to provide any reasons.
- I give permission to request information regarding my health, a hospitalization, or death from a medical centre, my physician, or the Central Bureau for Statistics, during or after the study.
- I give permission to link my donation information with the research information.
- I give permission to collect, use, and store my information and human tissue to answer the research questions in this study.
- I know that some persons will have access to all my information for study monitoring. These persons are mentioned in the information letter. I give these persons permission for access.
- I give permission to Sanquin to store my information and human tissue for 15 years after the study.
- I give permission to share my encrypted information and human tissue with domestic and international laboratories and researchers for purposes described in the information letter.

❖ I □ do

□ do not
give permission to collect DNA from my blood and to store and use is for this study

❖ I □ do

□ do not
give permission to approach my again for a potential follow-up study.

❖ I □ do

□ do not
give permission to store the human tissue, DNA, and research data for an additional 15 years, after the initial 15 years of storage as part of this study, so in total 30 years.

❖ I would like to participate in this study.

Name subject:……..

Donor number: ……...

Signature:…… Date: __ / __ / __

I declare that I have fully informed the subject about the afore mentioned study.

If information becomes available which could affect the informed consent, then the subject will be informed timely.

Name researcher (or the latter's representative): ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,……

Signature:…….. Date: __ / __ / __

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EIN-sticker:

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	x
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	19
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	19
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	x
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	x

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	17
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9, 13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9-10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	12
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	x
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14-15
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	x
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	15
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	x
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	17

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	17
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	17
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	19
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	x
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	17
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	x
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	x

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.