Protocol details

1.1 Protocol Title:

Randomised cross over study measuring iron bioavailability from oral iron supplements in healthy pre-menopausal women using stable isotopes

1.2 Protocol Version Control

Version number: *1.1*
Final/draft:
Date: 23/01/2024

1.3 Names (titles), roles and contact details:

Sponsor
Name of Sponsoring Organisation: King's College London
Name of Sponsor Representative: N/A
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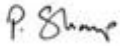
2 CI Signature

The Chief Investigator and the RGO (sponsor office) have discussed this protocol. The investigators agree to perform the investigations and to abide by this protocol.

The investigator agrees to conduct the study in compliance with the approved protocol, GCP, the UK General Data Protection Regulation and Data Protection Act (2018), the Sponsor's SOPs, and any other local or external regulatory requirements as required.

Chief investigator

Professor Paul A Sharp

Signature	Date
	23/01/2024

Contents

3 Summary/Synopsis

Full Title	Randomised controlled cross over study measuring iron bioavailability from oral iron supplements in healthy pre-menopausal women using stable isotopes
Short Title/Acronym	Bioavailability of iron supplements (BOIS study)
Protocol Version number and Date	1.0 13/11/2023
REMAS Number	
REC Name	Kings College London Research Ethics Committee
Study registration details (if applicable)	N/A
Chief Investigator	Professor Paul Sharp
Sponsor name	King's College London
Co-Sponsor *(if applicable)*	
Funder(s)	Nelsons & Co Ltd, Nelsons House, 83 Parkside, Wimbledon, London SW19 5LP
Population under investigation	Healthy pre-menopausal women aged 19-49 years with low iron stores (below 30 µg/L serum ferritin; normal range 30-100 µg/L).
Design & Methodology	Randomised controlled, cross-over study.
Study Duration	84 days for all interventions.
Primary objective	In this study we will compare iron absorption from 3 doses of iron (5, 10, 15 mg, given as either Spatone™ or ferrous sulphate. We hypothesize that at for each iron dose, absorption of iron will be greater from Spatone™ water than from ferrous sulphate tablets. In addition, we hypothesize that the fractional absorption of iron will be greatest at the lowest supplement dose.
Secondary objective(s)	N/A
Number of Participants	We expect to screen up to 40 volunteers to select 15 eligible volunteers
Endpoints	As each volunteer will act as their own control, the endpoint of the study will be after each volunteer completes all 6 interventions and provides a final blood sample two weeks following the final intervention
Main Inclusion Criteria	Healthy pre-menopausal women aged 19-49 years with low iron stores (below 30 µg/L serum ferritin; normal range 30-100 µg/L), but haemoglobin ≥ 120 g/L (i.e. not anaemic) will be recruited to the studies. Iron deficiency is prevalent in reproductive age women due to menstrual blood loss. Iron requirements decrease and iron stores increase in post-menopausal women. A lower age limit of 19 was chosen as this is the lower age limit for adults in the National Diet and Nutrition Survey (NDNS) rolling programme and we would like to relate our work to the NDNS data.
Main Exclusion Criteria	Exclusion criteria include: Allergy to iron supplements, pregnancy; post-menopausal women; a history of alcohol or substance abuse, reported history of CVD, diabetes, cancer, kidney, liver or intestinal disease, gastrointestinal disorder or use of drugs likely to alter gastrointestinal function, not donated blood recently (within the last 3 months prior to screening visit). Volunteers currently taking or planning to take any

	mineral or vitamin supplements during the course of the study will also be excluded.
Statistical Methodology and Analysis	Each participant will consume all tests preparations (i.e. 3 doses of Spatone™ and 3 doses of ferrous sulphate; 6 preparations in total). To permit analysis on between group and within group variations, data will be analysed using repeated measured ANOVA._
Human Tissue Samples *(if applicable)*	The blood samples collected will be processed on the day of collection and then frozen to store before analysis. The analysis will take place within 6 months of the end of the study and then the remaining plasma sample will be stored accordingly to analyse any other outcomes in the future (additional iron status biomarkers and inflammatory markers). Samples will be kept securely in the researchers' lab (Lab 3.109, Franklin−Wilkins Building, King's College London, 150 Stamford Street, SE1 9NH)
Data to be collected & associated storage arrangements *(if applicable)*	Personal data of participants including: name and signature, age, contact details, location data, gender and health data will be collected during screening. Data will be pseudonymised after analysis. Each participant's name will be replaced with a unique alphanumeric code. The actual health data collected, such as weight, height, age, blood test results, and dietary intake from the food diary, would be associated with this code and not the participant's name in the research database. Data will be stored in line with KCL Data retention Schedule.

4 Introduction

Pathologies associated with both iron deficiency and iron overload are highly prevalent in the UK and worldwide. Iron deficiency is the most common nutritional deficiency affecting 2 billion people globally and accounts for more than 50% of anaemia cases. While the majority of those affected live in low and middle income countries, up to 30% of women of childbearing age in the UK may also be iron deficient. In the National Diet and Nutrition Survey, 54% of females aged 11-18 years and 27% of women aged 19-50 years had iron intakes below the Lower Reference Nutrient Intake (LRNI); intake below this level is unlikely to satisfy the metabolic requirements for iron increasing the risk of iron deficiency. Thus maintaining good iron status has been highlighted as a public health priority in the UK for several “at risk” population groups.

It is estimated that the treatment of ailments associated with iron deficiency costs the NHS approximately £25 million per year. Conventional treatment for iron deficiency relies on supplements containing relatively high doses of iron (up to 65 mg iron / tablet). While these supplements improve iron status in the long term, they can cause gastro-intestinal disturbances and are therefore poorly tolerated by some patients. The challenge therefore is to develop alternative supplementation strategies which provides bioavailable iron without eliciting the adverse gastro-intestinal side effects. We have shown in an in vitro digestion model that iron absorption from Spatone™ is at least equivalent to that from ferrous sulphate (Christides T. et al., Eur J Nutr. 2015; 54(8):1345-52). Previous human volunteer studies have also shown that Spatone™ provides iron in a highly bioavailable form which is readily absorbed (Worwood M. et al., Clin Lab Haematol. 1996; 18(1):23-7; Halksworth G. et al. Clin Lab Haematol. 2003; 25(4):227-31).

These human studies calculated that iron absorption from Spatone™ may be higher than from ferrous sulphate tablets – up to 10% more. However, it is important to note that the volunteer group were **pregnant women** with very low serum ferritin levels and therefore a highly increased absorptive drive. This study will focus on **healthy non-pregnant** and pre-menopausal women aged 19-49 years with low iron stores (below 30 µg/L serum ferritin; normal range 30-100 µg/L).

Moreover, there is increasing evidence that consumption of higher doses of iron may adversely affect iron absorption from subsequent meals. For example, Moretti et al (Blood. 2015; 126(17):1981-9.) showed that absorption of iron from high-dose supplements, given on two consecutive days, was reduced significantly on day 2 by between 16-95%. This decrease in absorption correlated with both iron content of the supplement and an increase in plasma levels of hepcidin, the main iron regulatory peptide which acts as a negative regulator of iron absorption.

In summary, the evidence suggests that lower dose iron supplements such as Spatone™ may give rise to higher fractional iron absorption by having less impact on plasma hepcidin levels. Furthermore, there may be fewer gastro-intestinal side effects with lower dose supplements making this a more suitable strategy to maintain iron status is population groups at risk of iron deficiency.

5 Study/Trial objectives and purpose

5.1 Research hypothesis

The study is designed to comprehensively evaluate iron absorption by directly comparing the efficacy of Spatone™ with that of ferrous sulphate. It aims to gather dose-response information to understand how varying doses influence iron absorption. Additionally, the study seeks to investigate the impact of iron absorption on serum hepcidin levels, which is a significant factor as elevated hepcidin levels can inhibit both the absorption and utilization of iron. Understanding these dynamics is vital for improving treatments for conditions related to iron deficiency and for optimizing iron supplementation strategies. Three doses of iron either as Spatone™ or ferrous sulphate will be given to each participant. Our hypotheses are: 1. There will be a dose-dependent decrease in fractional iron absorption from both Spatone™ and ferrous sulphate preparations; 2. There will be no significant difference in iron absorption between Spatone™ and ferrous sulphate; 3. Spatone™ and ferrous sulphate will not affect hepcidin levels, even at the highest dose of each preparation used in this study.

5.2 Primary objectives

In this study we will compare iron absorption from 3 doses of iron (5, 10, 15 mg, given as either Spatone™ or ferrous sulphate). Our primary objective is to determine iron absorption from each preparation by measuring stable isotope (^{57}Fe) incorporation into haemoglobin.

5.3 Secondary objectives

The secondary objective of this study is to assess and compare the hepcidin response to a 15 mg dose of iron from two different supplements: Spatone™ and ferrous sulphate. Our starting hypothesis is that 15 mg iron from either source will not affect serum hepcidin levels. Hepcidin is a regulatory hormone that plays a key role in iron homeostasis. If hepcidin levels are increased by either iron source this would be an important finding as it would indicate a potential decrease in iron absorption from this supplemental source.

6 Study/Trial design & Flowchart

6.1 Study/Trial Design

This will be a Randomised controlled cross over study measuring iron bioavailability from oral iron supplement. In this study we will compare iron absorption from 3 doses of iron (5, 10, 15 mg, given as either Spatone™ or ferrous sulphate). Each volunteer will undergo six interventions, with a two-week interval between them. On the day of each intervention, a blood sample will be taken to measure either baseline levels of iron in haemoglobin or iron absorbed and incorporated into haemoglobin from the previous intervention administered two weeks prior.

For the interventions involving a 15 mg dose of iron, volunteers are required to make an additional visit to the Metabolic Unit at King's on the day following consumption of the supplement to provide an additional blood sample specifically for serum hepcidin measurement. This is critical to assess the body's hepcidin response to a higher dose of iron. For all other doses, the volunteers need only return two weeks after for their subsequent intervention.

During each visit, alongside the iron supplement, participants will also be asked to drink 100 mL mineral water containing 3 mg 57 Fe stable isotope.

6.2 Study/Trial Setting

The study will be conducted at the Metabolic Research Unit of the Franklin Wilkins Building, within the Department of Nutritional Sciences, KCL. The location is Corridor A, 4th Floor, Franklin Wilkins Building, 150 Stamford Street, London, SE1 9NH.

6.3 Flowchart

	Screening Visit (~40 volunteers)	Day 1	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
Participant information, informed consent and screening blood sample	x							
Blood Sample		x	x	x	x	x	x	x
5mg, 10mg, 15mg*, given as either Spatone™ or ferrous sulphate (6 interventions)		x	x	x	x	x	x	

** volunteers will visit MRU 24 hours after 15 mg intervention for hepcidin assay blood sample*

7 Participant selection

7.1 Participant inclusion criteria

Healthy pre-menopausal women aged 19-49 years with low iron stores (below 30 µg/L serum ferritin; normal range 30-100 µg/L). A series of questions regarding the study's inclusion characteristics will be completed by potential volunteers. Questionnaires will be posted/emailed to potential volunteers. The eligibility of each interested volunteer will be then confirmed during the screening visit where blood samples will be collected to assess iron status biomarkers. We will measure body weight, height and waist circumference according to standardised protocols (anthropometry).

7.2 Participant exclusion criteria

Exclusion criteria include: Allergy to iron supplements, BMI above 25; pregnancy; post-menopausal women; a history of alcohol or substance abuse, reported history of CVD, diabetes, cancer, kidney, liver or intestinal disease, gastrointestinal disorder or use of drugs likely to alter gastrointestinal function, not donated blood recently (within the last 3 months prior to screening visit). Volunteers planning to take any mineral or vitamin supplements during the course of the study will also be excluded. A series of questions regarding the study's inclusion characteristics will be completed by potential volunteers. Questionnaires will be posted/emailed to potential volunteers.

8 Study/Trial procedures

8.1 Participant recruitment

Method of recruitment:
Recruitment will be carried out via advertisements placed in local newspapers, and through the fortnightly Research Volunteer Recruitment emails sent to King's College London staff and students. Potential participants responding to these advertisements will be sent a screening questionnaire and study information sheet by post. Those passing the initial screen will be followed up by telephone interview and asked to attend an initial screening visit at King's College London to provide a blood sample for measurement of serum ferritin and haemoglobin.

Payment of participants:
Reimbursement of travel expenses (up to £10 per visit), inconvenience payment of £100 per volunteer, upon completion of the study. Volunteers who complete part of the study visits and withdraw from the study prior to the completion of the study will be reimbursed proportionally.

Gaining participant consent:
Interested volunteers will be sent a patient information sheet by post or email. If they are eligible according to the initial email screening questionnaire they will be invited to attend a screening session at KCL. At the screening visit it will be the responsibility of the qualified researcher to obtain written (signed and dated by the participant and researcher) informed consent from each individual participating in the study after adequate explanation of the aims, methods, objectives and potential hazards of the study. The researcher will also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time and volunteers will have the opportunity to ask questions. The participant will be provided with a copy of their signed and dated consent form and any other written information which they should be instructed to retain. Screening results will only be given to the volunteer's GP if results are outside the normal range and the volunteer wishes it. In this case the volunteer will be supplied with a letter to pass on to their GP.

Potential participants will be able to discuss the study and read the information leaflet for at least 24 hours prior to a screening appointment, booked at the participant's convenience. They will be asked to sign the consent form at the screening visit. They will be informed over the phone or via email, prior to the screening visit, that they are free to withdraw from the study at any point and they are not obliged to give a reason. At the screening visit it will be the responsibility of the qualified researcher to obtain written (signed and dated by the participant and researcher) informed consent from each individual participating in the study after adequate explanation of the aims, methods, objectives and potential hazards of the study, prior to making any measurements or collecting any blood samples.

8.2 Screening Procedures

Interested volunteers will be sent a participant information sheet by post or email. If they are eligible according to the initial email screening questionnaire they will be invited to attend a screening session at KCL. At the screening visit it will be the responsibility of the qualified researcher to obtain written (signed and dated by the participant and researcher) informed consent from each individual participating in the study after adequate explanation of the aims, methods, objectives and potential hazards of the study. The researcher will also explain to the subjects that they are

completely free to refuse to enter the study or to withdraw from it at any time and volunteers will have the opportunity to ask questions. The participant will be provided with a copy of their signed and dated consent form and any other written information which they should be instructed to retain. For screening, a trained phlebotomist will draw 17 mL of blood from the participant. Screening results will only be given to the volunteer's GP if results are outside the normal range and the volunteer wishes it. In this case the volunteer will be supplied with a letter to pass on to their GP. Participants will be informed within a week of screening about their eligibility.

We will ensure that all participants that undergo screening are logged into a screening log associated with the study/trial.

8.3 Randomisation Procedures (if applicable)

Once we have our 15 eligible volunteers, we will employ a simple randomization approach using Microsoft Excel. This method involves assigning each volunteer a unique identifier, then using Excel's random number generation function to assign a random number to each identifier. These numbers are then sorted, and the list is ordered based on these random numbers. The list will determine the order in which the preparations will be consumed.

8.4 Blinding & other measures taken to avoid bias – N/A

8.4.1 Blinding N/A

8.4.2 Other measures taken to minimise/avoid bias N/A

8.5 Intervention Procedures

The intervention procedure is structured to ensure a detailed assessment of iron bioavailability. Each volunteer will undergo six interventions, with a two-week interval between them. Participants will be asked to fast overnight (starting 9 pm) before the day of intervention. On the day of each intervention, a blood sample will be taken to serve two purposes: it will provide a baseline for the iron supplement about to be administered and offer insight into the bioavailability of the previous intervention administered two weeks prior.

For the interventions involving a 15 mg dose of iron, volunteers will be required to make an additional visit the day after consumption of the supplement to provide a blood sample specifically for hepcidin measurement. This is critical to assess the body's hepcidin response to a higher dose of iron. For all other doses, the volunteers need only return two weeks after for their subsequent intervention.

During each visit, alongside the iron supplement, participants will also be asked to consume 100 mL mineral water containing 3 mg 57 Fe stable isotope as a tracer. This tracer is pivotal in determining

the bioavailability from each intervention. Nelsons and Co, with their GMP and UKAS accredited laboratory, will prepare all solutions. These are commercial-grade products already available at major high street retailers including Boots, Waitrose, and Superdrug.

8.5.1 Substance/Products to be Administered

Product details: Spatone™
Manufacturer: Nelsons & Co Ltd
Supplier: Nelsons & Co Ltd
Storage conditions: *Cool dry place at or below 25°C.*
Dosing regimen: 5 mg, 10 mg, 15 mg
Duration of participant use: Single use

Spatone™ will be supplied pre-prepared in sachets containing 5 mg iron. Participants will be asked to consume (oral route) 1, 2 or 3 sachets of Spatone™ (i.e., 5, 10 or 15 mg iron). Maximum dose of iron consumed corresponds to 101% of RNI for females aged 19-50 years. Consumption of each preparation will be supervised by study staff.

Product details : Ferrous Sulphate Capsules
Manufacturer: Nelsons & Co Ltd
Supplier: Nelsons & Co Ltd
Storage conditions: *Cool dry place at or below 25°C.*
Dosing regimen: 5mg, 10mg, 15mg
Duration of participant use: Single use

Each capsule will contain 5mg of ferrous sulphate. Volunteers will consume (oral route) either one capsule (5 mg), 2 capsules (10 mg) or 3 capsules (15 mg). Maximum dose of iron consumed will be 101% of the RNI for females aged 19-50 years. Ferrous sulphate for therapeutic use is normally supplied as 65 mg iron dose. Commercially available iron supplements from UK high street retailers contain up to 20 mg iron. Therefore, we do not expect any adverse reactions between 5 mg and 15 mg doses.

Storage – the iron supplements will be stored in a cool dry place at or below 25°C. There is a secure food storage unit on 4th floor FWB. This is adjacent to the Metabolic Research Unit and the materials will be stored here prior to use in the studies. This is a food storage unit and the room temperature is monitored regularly.

8.6 End of Study/Trial Definition

As each volunteer will act as their own control, the endpoint of the study will be after each volunteer completes all 6 interventions and provides a final blood sample two weeks following the final intervention.

9 Laboratories

Screening blood samples will be assessed at Affinity Biomarkers Laboratories (refer to page 2). Remaining bloods samples will be processed on the day of collection and then frozen to store before analysis. Stable isotope incorporation will be measured by our collaborators at Atomic & Mass Spectrometry unit, Department of Chemistry, Ghent University, Belgium. We will extend our current Material Transfer Agreement to cover samples collected in the current study. All subsequent analysis will be carried out by an experienced researcher at King's College London.

Researchers involved in this study will undertake HTA training.

9.1 Central/Local Laboratories

Affinity Biomarkers Laboratories (refer to page 2)

9.2 Sample Collection/Labelling/Logging

All blood samples will be taken by a trained phlebotomist. The blood samples collected will be processed on the day of collection and then frozen to store before analysis.

9.3 Sample Analysis Procedures

Whole blood samples collected as part of this study will be used to measure stable isotope incorporation into haemoglobin as a measure of iron bioavailability. This analysis will be carried out by our partners at Ghent University. Measurement of stable isotopes requires specialist equipment, specifically a multi-collector inductively coupled plasma mass spectrometer, which is not available at King's College London.

Serum samples will be used to measure serum hepcidin levels and C-reactive protein levels as markers of iron status and inflammation, respectively. Both analytes will be measure using commercially available ELISA kits.

9.4 Sample Storage Procedures

Processed samples will be stored in a HTA registered -80 C freezer in FWB, King's College London.

9.5 Results Recording/Reporting

Results will be recorded on Microsoft Excel and statistical analysis software, e.g. GraphPad Prism, will be used for further statistical analysis.

9.6 Sample Receipt/Chain of Custody/Accountability

N/A

9.7 Sample Transfer to sites outside the Organisation

Blood samples will be transferred to our collaborators at Atomic & Mass Spectrometry unit, Department of Chemistry, Ghent University, Belgium for measurement of stable isotope incorporation into haemoglobin. We have an existing Material Transfer Agreement which permits the transfer of biological samples for this purpose. Blood samples from the study will be collected in duplicate and one of the samples will be retained at King's in case of any problems during the transfer of material. The samples sent to Ghent University will not be used for any purpose other than analysis of stable isotopes in haemoglobin and the samples will be destroyed once the analysis is completed.

9.8 Sample storage for ancillary use

The blood samples collected (in duplicate) will be processed on the day of collection and then frozen to store before analysis. The analysis will take place within 6 months of the end of the study and then the remaining plasma sample will be stored accordingly to analyse any other outcomes in the future (additional iron status biomarkers and inflammatory markers).

10 Approvals

The study protocol and other documentation will be submitted to the King's College London Research Ethics Committee before the start of the study.

All correspondence with the REC will be retained.

Any amendments to the study will be submitted to the King's College London Research Ethics Committee as a modification request through REMAS. Amendments will not be implemented until a favourable opinion is issued.

11 Safety & Adverse Events Reporting

11.1 Assessment of Safety

To ensure participant safety throughout the study, several measures will be in place. Trained phlebotomists will be responsible for drawing blood, minimizing the risk associated with blood sample collection. The Metabolic Research Unit (MRU) has an in-house trained paramedic available to handle any immediate medical issues that may arise during the visits.

After the collection of each blood sample, all volunteers will be closely monitored for any signs of adverse reactions or discomfort before they are allowed to leave the MRU.

In the event of any adverse occurrences, these will be reported immediately to the Research Governance Office.

12 Compliance and withdrawal

12.1 Participant compliance

Volunteers will be provided with a 3-day diet diary to record their food and beverage intake for two weekdays and one weekend day prior to the study visits.

12.2 Withdrawal / dropout of participants

Withdrawal from the study will be dealt with face-to-face or over the telephone if possible (otherwise by email or letter) so that the participant can confirm withdrawing from the study and express any concerns regarding the study if they wish. If they withdraw following screening but before attending the first study visit, their data will be not be used in the study. If they withdraw once the study has started their data will be used in the final report unless the participant requests the withdrawal of their data. Participants will be informed that their data cannot be withdrawn once the study has been published in a journal or compiled in a report, and that any data that is collected after the point of randomisation will be anonymised. Should a subject decide to withdraw all efforts will be made to complete and report the observations as thoroughly as possible. If a participant does withdraw, an explanation of why the subject is withdrawing from the study will be recorded. If a participant withdraws, consent will be obtained to use the previously collected data If the reason for removal of a subject from the study is an adverse event or an abnormal laboratory test result, the principal specific event or test will also be recorded on the personal file. Any adverse events ongoing at the final visit which are considered in any way related to the study treatment or study regime will be followed up until resolved, the condition stabilises or is otherwise explained. Subject Replacement: Subjects will not be replaced on the study in the event of subject withdrawal unless the study has not yet commenced or is in its very early stages. In order to avoid loss of statistical power through subject withdrawal, an adequate sample size will be conserved by initially recruiting 30% more subjects than required.

12.3 Protocol Compliance

Any instances of non-compliance with the study protocol will be meticulously documented and reported to the Principal Investigator (PI) in a timely manner. The PI will then relay this information to the Sponsor as per regulatory requirements. A protocol deviation log will be maintained to record any discrepancies from the study plan. This log will be regularly reviewed to identify any patterns of non-compliance and to facilitate immediate corrective action, ensuring that recurrent deviations, which may be classified as serious breaches, are addressed promptly. Given the straightforward nature of this intervention study, where all six interventions are administered directly by an experienced researcher, the likelihood of non-compliance is anticipated to be minimal. Additionally, the design of the study allows volunteers to resume their normal daily activities immediately after consuming the intervention, which further reduces the potential for non-compliance.

13 Data

13.1 Data to be collected

The data collection for this study will involve the following components:

Weight and Height: This information will be collected from the screening questionnaire filled out by participants. This data serves as part of the eligibility criteria and for calculating body mass index (BMI).

Age: Also collected from the screening questionnaire, age is a vital demographic factor that may influence the study's outcomes.

Iron Status Indicators: The baseline blood sample, taken prior to the first intervention, will establish the participant's iron status before any treatment is administered. This includes, but is not limited to, serum ferritin, haemoglobin, and transferrin saturation.

Post-Intervention Iron Status: Subsequent blood samples, taken two weeks after each intervention will be analysed to assess iron absorption from each supplemental iron preparation.

Hepcidin Levels: An additional blood sample will be collected the day after the administration of the 15 mg iron dose to assess the serum hepcidin response to high-dose iron from both ferrous sulphate and Spatone™.

The source of the data for weight, height, and age will be the participant questionnaires. Blood samples will provide the remaining data, which will be obtained through venipuncture by a trained phlebotomist.

Time points for data collection will include:

Baseline data from questionnaires at screening.
Baseline iron status prior to each intervention.
Follow-up iron status two weeks after each intervention, and the hepcidin response 24 hours after the 15 mg dose.
The data will be collected by trained research staff.

To maximize the completeness of the data, particularly from participant questionnaires, reminders via telephone or electronic communication may be used.

13.2 Data handling and record keeping

Participants' personal details will be kept securely (locked cabinets) in the researchers' office (Room 3.108, Department of Nutritional Sciences, Franklin–Wilkins Building, King's College London, 150 Stamford Street, SE1 9NH). Personal data stored in filing cabinets, cupboards and/or rooms will be kept in a locked room when not in use. Personal data held within computers will be password protected and stored on encrypted memory sticks or restricted server access when not in use. Access to such data will be granted only to appropriate members of the research team. On completion of the study, the research project's paper records will be stored in a secure

environment (filing cabinet in a locked office) that enables continued access to the required records by the researcher team and authorised members of the department.

Anonymised data from the study will be stored for at least 10 years as they can be used for purposes such as calculation of statistical power for future studies or for teaching purposes.

13.3 Access to the dataset

Dr Mohamad Farshard Aslam (postdoctoral research associate working on this project) and Professor Paul Sharp (PI) will have access to this dataset during the study. Upon completion of the study, Nelsons & Co Ltd may access the anonymised data analysed by the researchers.

14 Statistical considerations

Professor Paul Sharp and Dr Mohamad Farshard Aslam were involved in the study design and carried out the sample size calculations.

14.1 Sample size calculation

The study has been powered using data from previous human intervention studies measuring changes in iron absorption in volunteers with reduced iron stores. Iron absorption in volunteers consuming a test meal containing 20 mg iron was 12.6% (Wawer A. et al., PLoS ONE 9(11): e112144). Similarly, absorption from meals containing 6 mg or 60 mg iron was approximately 20.4% and 10.0%, respectively (Brittenham G. et al., Am J Clin Nutr 2014;100:813–20). Based on these data we estimate that absorption from supplements containing 5, 10, or 15 mg iron will be 20%, 16% and 14%, respectively with a pooled standard deviation of ± 6.5% and η^2 of 0.83. Based on these data we have calculated (using G*Power sample size calculator) that we would need 5 participants to detect statistical differences between doses of Spatone™ and 5 participants to detect statistical differences between doses in the ferrous sulphate group, in both cases with 80% power and $\alpha = 0.05$. While participants will consume both supplemental iron forms, and we do not expect to see significant differences in iron absorption between Spatone™ and ferrous sulphate, we will none the less take a conservative approach to sample size. We will therefore recruit 10 volunteers to the study – this will be sufficient to detect statistical differences in iron absorption between Spatone™ and ferrous sulphate should they occur. Previous studies indicate that the volunteer drop-out rate could be up to 30%. We will therefore aim to recruit 15 volunteers to the study. Based on cut-offs for serum ferritin in the UK NDNS, we estimate that approximately 40% of the UK female population aged 19-50 years will have serum ferritin below 30 µg/L. Therefore, we will need to screen a maximum of 40 volunteers to achieve our recruitment target.

14.2 Statistical analysis

Iron absorption will be measured from 57 Fe stable isotope incorporation into haemoglobin. Data for each dose of Spatone™ and ferrous sulphate will be presented as the geometric mean and interquartile range, and the 95% confidence intervals will also be calculated. Statistical analysis of the absorption data will use repeated measures ANOVA with post hoc pairwise comparisons (Bonferroni test) where required.

The secondary outcome of the study is to measure serum hepcidin levels in participants consuming 15 mg iron either as Spatone™ or ferrous sulphate. Baseline serum hepcidin will be measured from blood samples taken prior to consumption of 15 mg iron dose and post-treatment levels measured in blood samples taken the following day. Data will be presented as median and interquartile range, and the 95% confidence intervals will also be calculated. Data will be analysed separately for each group (i.e. Spatone™ or ferrous sulphate) using Wilcoxon Signed Ranks Test for paired data.

14.3 Interim analysis and data monitoring

14.3.1 Stopping/ discontinuation rules and breaking of randomisation code

Sample preparation for iron bioavailability analysis will take place after volunteers complete all six interventions. Therefore, randomisation codes will not need to be broken.

14.3.2

N/A

15 Ethical considerations

N/A

16 Risk Management & Safeguarding

16.1 Risk Management

Venepuncture can cause brief discomfort and there is a risk of bruising. Efforts will be made to minimise this risk by ensuring that all blood samples will be taken by a trained phlebotomist. The blood samples collected will be processed on the day of collection and then frozen to store before analysis. Spatone™ will be administered as an orally in liquid form. Volunteers may choke if the substance is not administered appropriately (i.e. volunteer not sitting upright while ingesting liquid). In order to minimise this risk, volunteers will be asked to sit upright and ingest the liquid slowly. Ferrous sulphate will be administered as tablets. Volunteers may choke/gag if the tablets are not administered appropriately (i.e. water not provided along with tablets). In order to minimise these risks, volunteers will be provided with plenty of water along with the tablets and will be asked to sit upright while ingesting the tablets. Most over the counter multivitamins contain approximately 14 - 20 mg iron. The highest dose of iron administered in this study will be 15 mg, therefore, we would not expect any gastro-intestinal disturbances at the administered doses of 5 mg, 10 mg or 15 mg.

There are first aiders available via KCL's internal phone system. Beyond that, the Metabolic Research Unit is managed by a registered paramedic and the unit has its own defibrillator and paramedic drugs aimed at managing the medical emergencies most likely to be encountered at the research unit, including anaphylaxis. Regarding fainting specifically, all who perform venepuncture are instructed in understanding and managing fainting (including practical demonstrations and role play), are given a handout to study and keep, and are shown how each phlebotomy chair works. In addition, each phlebotomy room has a laminated emergency card in situ providing a step-by-step guide to standard procedures. The venepuncture SOP is explicit in ensuring that venepuncture is never carried out by a lone worker and that all participants with low bp and/or low resting heart rates and/or previous experience of fainting are laid down prior to the procedure being carried out, thus negating the risk as far as possible.

16.2 Safeguarding

Research team will not be working with children and/ or vulnerable adults.

17 Financing

Research funding provided by Nelsons & Co Ltd, Nelsons House, 83 Parkside, Wimbledon, London SW19 5LP.

18 Insurance

The study is sponsored by King's College London (KCL). KCL through its own professional indemnity (Clinical Trials) & no-fault compensation, in respect of any claims arising as a result of negligence by its employees, brought by or on behalf of a trial participant.

19 Publication and Dissemination

19.1 Publication

Following the study, all named investigators on the application will have access to raw data and right to publish. Nelson & Co Ltd. (Funders) have agreed that we can publish the results regardless of the outcome of the study. This agreement is included in the contract between KCL and Nelson & Co Ltd.

19.2 Informing participants

All participants will receive an email link to any publications related to this study.

20 Disclosure of Interests

None