Clinical Study Protocol

Impact of ferric citrate vs ferrous sulfate on iron parameters and hemoglobin in individuals with moderate to severe chronic kidney disease (CKD) with iron deficiency

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1 Background and Rationale

Disturbances in iron homeostasis and bone/mineral metabolism are common complications of chronic kidney disease (CKD) that lead to a number of adverse outcomes including anemia, bone disease and cardiovascular mortality.[1] In clinical practice, disorders of iron homeostasis and bone/mineral metabolism are considered distinct entities and are treated separately. However, emerging evidence suggests that hormones regulating bone/mineral metabolism and iron homeostasis interact with each other on multiple levels, and that taking account of these interactions will improve the treatment of both disorders of iron homeostasis and disturbances in bone/mineral metabolism.

Iron plays a crucial role in the physiology of oxygen transport. Excess iron can lead to oxidative stress, while its deficiency can lead to anemia and poor oxygen delivery. Tight control of iron metabolism is required for normal physiological function. Since humans lack the ability to actively excrete excess iron, iron absorption is tightly controlled at the proximal small intestine and within the reticuloendothelial system.[2] Hepcidin is the master regulator of iron metabolism.[3] Hepcidin regulates iron metabolism by binding to the cell-surface iron transporter, ferroportin, resulting in its internalization and lysosomal degradation.[4] This effectively blocks iron absorption in the gut and iron release from macrophage and hepatocytes resulting in decreased iron availability for erythropoiesis.[4,5] States of increased iron demand such as iron deficiency lead to a fall in hepcidin levels and thus increased iron absorption.[3,6] Alternatively, in states of inflammation (such as CKD) or iron overload, hepcidin concentrations are elevated leading to decreased iron availability for erythropoiesis.[3,6]

Iron deficiency is highly prevalent in CKD and is a major mechanism underlying anemia of CKD.[7,8] It can be broadly classified as either absolute—in which total iron stores are depleted—or functional, in which iron stores are sufficient, but unable to be mobilized from the reticuloendothelial system (RES) for erythropoiesis. Hepcidin concentrations are elevated in CKD and are a key mechanism underlying anemia of CKD.[1] Experimental data show that reducing hepcidin improves anemia of CKD,[9-11] suggesting that interventions that mitigate the rise in hepcidin can help to reduce the prevalence and severity of anemia in CKD patients.

Individuals with CKD respond poorly to oral iron relative to individuals without kidney disease. One major reason for this is that iron supplementation stimulates hepcidin secretion in CKD patients, who already have constitutively elevated hepcidin concentrations, further exacerbating iron-restricted erythropoiesis. For this reason, mitigating the rise in hepcidin with iron supplementation in individuals with CKD could be highly advantageous in that it would allow for better iron bioavailability (improvement in serum iron parameters) by enhancing gut iron absorption while at the same time attenuating iron sequestration within the RES. In addition to treating anemia of iron deficiency, this could enhance responsiveness to erythropoietin stimulating agents which require iron mobilization from the RES for maximum efficacy.[3] Pharmaceutical agents that can both increase iron stores and mitigate the rise in hepcidin secretion following iron supplementation are not currently available.

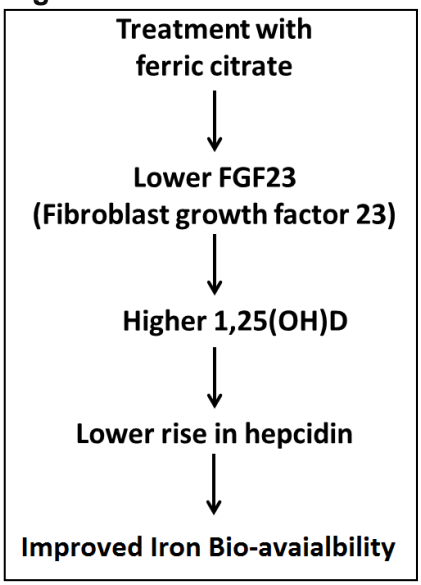
Low vitamin D concentrations are independently associated with increased prevalence and severity of anemia.[12-15] The proposed mechanisms by which vitamin D influences erythropoiesis have largely focused on the inhibitory effects of vitamin D on inflammation and its stimulatory effects on erythropoiesis.[16] However, recent data suggest vitamin D may also impact erythropoiesis by modulating iron homeostasis via regulation of hepcidin synthesis. Bacchetta et al[17] showed that 1,25-dihydroxyvitamin D directly inhibits hepcidin expression by binding to a vitamin D response element in the gene encoding hepcidin (HAMP). Further, in a small study

healthy volunteers, treatment with a single oral dose of ergocalciferol (100,000 IU) reduced hepcidin by 34% within 24 hours, which was sustained at 72 hours.[17] Another study showed that nutritional vitamin D supplementation was associated with reduced hepcidin concentration in individuals with stage 3 or 4 CKD.[18] Together, these data indicate that vitamin D is a potent inhibitor of hepcidin expression and increasing 1,25-dihydroxyvitamin D can lower hepcidin concentrations in individuals with CKD.

An important mechanism underlying the high prevalence of 1,25-dihydroxyvitamin D in CKD is elevated fibroblast growth factor 23 (FGF23) concentrations. FGF23 is a hormone secreted by bone cells that regulates vitamin D and phosphorus homeostasis.[19] FGF23 enhances urinary phosphorus excretion and decreases the activation of vitamin D by inhibiting 1-alpha-hydroxylase, the enzyme which converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. Elevated circulating concentrations of FGF23 are extremely common in CKD, likely as a physiological adaptation to help maintain serum phosphorus in the normal range by enhancing urinary phosphorus excretion and reducing gut absorption of phosphorus via inactivation of vitamin D.[19] While this is important for maintaining phosphorus balance in the short term, this has long-term consequences such as progressive reductions in the synthesis of 1,25-dihyroxyvitamin D.

Ferric citrate effectively binds dietary phosphorus while improving iron status and hemoglobin in individuals with CKD.[20] In addition, ferric citrate has been shown to reduce FGF23 concentrations in individuals with pre-dialysis CKD.[20] Based upon these data, we hypothesize that in individuals with moderate to severe CKD, as compared to standard oral iron formulations used to treat iron deficiency anemia, treatment with ferric citrate will more effectively improve iron parameters than ferrous sulfate, in part by mitigating the rise in hepcidin concentrations following iron repletion by reducing FGF23 concentrations and enhancing vitamin D activation. Thus, ferric citrate will be shown to be unique in that it can improve iron parameters and hemoglobin while at the same time mitigating iron-induced stimulation of hepcidin secretion, providing superior short- and long-term effects on iron-restricted erythropoiesis in CKD (**Figure 1**).

Figure 1



1.1 Study Rationale

Ferric citrate is an FDA-approved oral phosphorus binder that has been shown to be effective in reducing serum phosphorus and FGF23 concentrations and increasing iron stores and hemoglobin in individuals with non-dialysis-dependent CKD who have iron-deficiency anemia.[20] This may prove to be advantageous in individuals with pre-dialysis CKD who require iron supplementation for iron-deficiency anemia. This is because ferric citrate may not only restore iron stores in individuals who are iron deficient, but by lowering FGF23 concentrations, ferric citrate may increase local and systemic concentrations of 1,25-dihydroxyvitamin D, a powerful inhibitor of hepcidin synthesis, potentially attenuating the increase in hepcidin following oral iron supplementation. When compared to standard iron supplementation therapies (e.g., oral ferrous sulfate) that powerfully stimulate hepcidin secretion, this may then allow for greater iron bioavailability by increasing iron absorption in the gut while also reducing the degree of iron sequestration in RES stores. However, little is known about the comparative effectiveness of treatment with oral ferric citrate vs. oral ferrous sulfate (currently the standard of care) in increasing iron stores and hemoglobin in iron-deficient CKD patients. If ferric citrate is shown to not only improve overall iron status, but also partially mitigate the long-term effects of iron

supplementation on hepcidin secretion by increasing endogenously produced 1,25-dihydroxyvitamin D, this may indicate that ferric citrate can provide superior short- and long-term effects on iron-restricted erythropoiesis in CKD as compared to the current standard of care.

2 Study Objective and Hypotheses

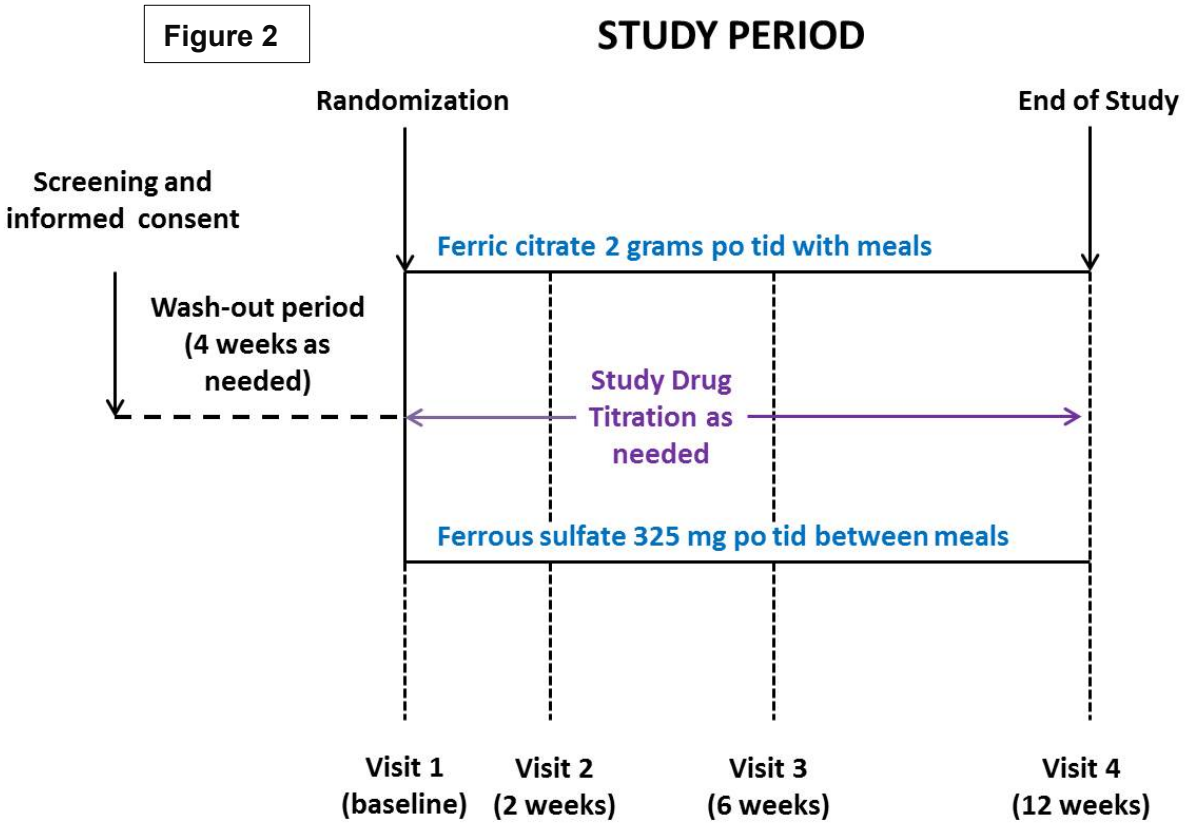
The main objectives of the study are to compare the impact of ferric citrate compared to standard of care ferrous sulfate on serum iron, percent transferrin saturation (TSAT), ferritin, hemoglobin and hepcidin concentrations in individuals with moderate to severe CKD and absolute iron deficiency. We will test the following hypotheses:

Primary Hypothesis: In patients with moderate to severe CKD not requiring dialysis (estimated glomerular filtration rate 15-45 ml/min/1.73m^2) with absolute iron deficiency (serum ferritin ≤300ng/ml and TSAT ≤ 30%), treatment with ferric citrate will lead to greater improvements in markers of iron bioavailability (serum iron, ferritin, transferrin saturation) than treatment with ferrous sulfate.

Secondary Hypotheses: In patients with moderate to severe CKD not requiring dialysis (estimated glomerular filtration rate 15-45 ml/min/1.73m^2) with absolute iron deficiency (serum ferritin ≤300ng/ml and TSAT ≤ 30%), as compared to treatment with ferrous sulfate for 12 weeks, treatment with ferric citrate for twelve weeks will result in (1) a greater rise in hemoglobin concentrations and (2) a lower rise in serum hepcidin concentrations.

3 Investigational Plan (Figure)

Figure 2



3.1 General overview

- Randomized, open-label study of ferric citrate vs. ferrous sulfate in patients with moderate to severe CKD (eGFR 15 – 45 ml/min/1.73m^2).
- Participants will be recruited from the outpatient nephrology clinics at the University of Alabama at Birmingham (UAB).
- Written informed consent will be obtained at the screening visit after the study has been explained in full.
- Participants taking oral iron supplements at the time of the screening visit will undergo a four week wash-out period prior to beginning study medications.
- After eligibility is confirmed following the screening visit (and wash-out period if necessary), participants will be randomly assigned 1:1 to take ferric citrate at a dose of 2 grams three times a day with meals (n=30) or ferrous sulfate at a dose of 325 mg by mouth three times a day between meals (n=30) for a total of twelve weeks.
- Fasting blood and urine samples will be collected at baseline and two, six and 12 weeks after baseline for measurement of study outcome variables. Data for the outcome variables as well as safety will be collected at these time points.
- The study will end at 12 weeks, a final blood and urine sample will be drawn and intervention will be stopped.

3.2 Study Population

3.2.1 Participant Recruitment

Participants will be recruited via several different methods:

- Outpatient clinic visits: Study staff will pre-screen patients coming in for their scheduled appointments at the Kirklin Clinic (TKC), the major outpatient nephrology clinic at UAB.
- Advertisements: Advertisements will be posted in local newspapers and online resources to recruit individuals with a history of kidney disease from the city of Birmingham and surrounding communities. Study flyers will be posted around the UAB hospital and outpatient clinic locations. These strategies have been successful in recruiting participants with CKD in randomized, controlled studies of CKD patients at our center.

3.2.2 Inclusion Criteria

- 18 years of age or older
- Moderate to severe kidney disease not requiring renal replacement therapy (eGFR 15-45 ml/min/1.73m^2 as determined by the CKD-EPI formula).
- Absolute iron deficiency as defined as a transferrin saturation ≤ 30% and a ferritin ≤ 300 ng/ml.

3.2.3 Exclusion Criteria

- Hemoglobin concentrations > 13 g/dL
- Severe anemia defined as a hemoglobin < 8.0 g/dL for males or a hemoglobin <7.0 g/dL for females.
- Known disorder of iron homeostasis (e.g., hemochromatosis)
- Known gastrointestinal disorder (irritable bowel disease, inflammatory bowel disease)
- Known or biochemical evidence of liver disease (ALT/AST or bilirubin > 3x normal)
- Serum phosphorus concentrations < 3.0 mg/dL
- Any known cause of anemia other than iron deficiency or CKD (e.g., sickle cell anemia)
- Symptomatic gastrointestinal bleeding within 12 weeks prior to the screening visit.
- Subjects receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis, or renal transplant.
- Pregnancy or lactation in female participants
- Life expectancy less than 6 months
- Receipt of erythropoiesis stimulating agents within 4 weeks of screening.
- Receipt of intravenous iron therapy within 8 weeks of screening.
- Blood transfusion within 4 weeks of screening
- Known allergies or severe adverse reactions to previous oral iron therapy
- Current use of oral phosphorus binders
- Current use of an active vitamin D analog
- Concomitant participant in another clinical study with investigational medicinal products.

3.2.4 Concurrent use of oral iron supplements

Potential participants taking oral iron (either as an over-the-counter supplement or prescribed medication) will not be excluded but will be required to stop the iron for at least 4 weeks prior to entering the study.

3.2.4.1 Concurrent use of other medications

All concurrent medications will be recorded in study participants. If participants are taking oral calcium supplements or nutritional vitamin D analog, they will be allowed to continue to take them as long as the dose does not change during the course of the study.

3.3 Justification of selection criteria

The selection criteria were chosen to focus enrollment on individuals with moderate to severe CKD (eGFR 15 – 45 ml/min/1.73m^2) who are iron deficient and (1) would not be exposed to particular risks from the study drug and (2) would not have conditions that would impact the aims of the study. The reason why we chose this cut-off for eGFR is that we wanted to enroll individuals who would benefit the most from an intervention that not only provided supplemental iron but also could potentially help to attenuate the rise in hepcidin following iron supplementation by reducing FGF23 concentrations. While it is possible that CKD patients with a higher eGFR would also benefit from using ferric citrate to increase iron stores, these individuals would likely have milder forms of iron deficiency and/or mildly increased FGF23 concentrations, making it more difficult to ascertain whether a therapy that combined iron repletion with phosphorus lowering would more effectively improve iron bioavailability than individuals with more severe forms of kidney disease. We chose not to include individuals with an eGFR < 15 ml/min/1.73m^2 because in our experience, it would be very challenging to find individuals at this low an eGFR who would be eligible for enrollment given our exclusion criteria—particularly avoidance of ESA exposure—within the period of time for recruitment proposed for this study (2 years). Furthermore, these individuals are more likely to be hospitalized and/or progress to ESRD requiring dialysis, which would increase the drop-out rate and require us to either extend the recruitment period or enroll a larger sample size than currently planned.

3.4 Study Schedule (Figure 2)

3.4.1 Screening Visit

Written, informed consent will be obtained by study staff during the screening visit after the study has been explained in full. Participants will be instructed to come to the screening visit after at least an 8-hour fast. Participants who provide consent will have height and weight obtained. Age, gender and race/ethnicity data will be recorded. Blood samples will be drawn to measure screening labs to ensure eligibility. In addition, female subjects of child-bearing age (≤ 55 years) will have a rapid urine test for beta-HCG performed to rule out pregnancy. Eligibility to continue with the study will be determined after review of screening tests. If any screening laboratory values are found to disqualify the participant, he or she will be informed of the findings, and will be asked to follow-up with his or her primary care physician or nephrologist for further evaluation if necessary (e.g., for a very low hemoglobin level).

Participants found not to be eligible following the screening visit and lab testing will be allowed to re-screen at a later time provided that the major reason for screening failure can be reasonably expected to be resolved within six months of the original screening visit (for example, a CKD patient who received IV iron three weeks ago, but who will refrain from IV iron within the next six months may be eligible for re-screening at a later date).

3.4.2 Washout Period

Participants who are currently receiving oral iron ferrous sulfate (or other oral iron preparations) will undergo washout of these medications for four weeks.

3.4.3 Visit 1 (Day zero)

After eligibility is confirmed in the screening visit and following the washout period if necessary, participants will come to the UAB Clinical Research Unit (CRU) in the morning after an overnight fast for visit 1(Day 0). During this visit, participants will undergo fasting blood collection to

establish baseline measurements for primary outcome variables (iron, TIBC, ferritin), secondary outcome variables (hemoglobin, hepcidin, FGF23) and safety measures (eGFR, calcium, phosphorus). At this time subjects will also be randomly assigned to a treatment arm (ferric citrate vs. ferrous sulfate).

3.4.3.1 Randomization (Visit 1)

All eligible participants will be randomized by the research pharmacist in a 1:1 fashion into one of two treatment arms:

- Ferrous sulfate at a dose of 325 mg by mouth three times a day between meals
- Ferric citrate at a dose of 2 grams three times a day with meals

3.4.4 Visit 2 (week 2)

- Participants will come to the UAB CRU, where participants will undergo vital sign measurement and fasting blood collection for measurement of study outcome variables and safety measures.
- Pills remaining in the study medication bottles of each participant will be counted to ensure compliance and accountability for discrepancies in study drug use will be performed.
- Participants will be asked questions about intercurrent medical events and changes in clinical condition from the last visit. Events that meet the definition of an adverse event (see section 3.9.2) will be documented.

3.4.5 Visit 3 (week 6)

- Participants will come to the UAB CRU, where participants will undergo vital sign measurement and fasting blood collection for measurement of study outcome variables and safety measures.
- Pills remaining in the study medication bottles of each participant will be counted to ensure compliance and accountability for discrepancies in study drug use will be performed.
- Participants will be asked questions about intercurrent medical events and changes in clinical condition from the last visit. Events that meet the definition of an adverse event (see section 3.9.2) will be documented.

3.4.6 Visit 4 (week 12)

- Participants will come to the UAB CRU, where participants will undergo vital sign measurement and fasting blood collection for measurement of study outcome variables and safety measures.
- Pills remaining in the study medication bottles of each participant will be counted to ensure compliance and accountability for discrepancies in study drug use will be performed.
- Participants will be asked questions about intercurrent medical events and changes in clinical condition from the last visit. Events that meet the definition of an adverse event (see section 3.9.2) will be documented. At the end of this visit, the study will be completed.

3.5 Study Drug Dispensing and Logistics

3.5.1 Study drug dispensing

The UAB research pharmacy will be responsible for storage of all study drugs in a secure facility. They will also be responsible for procurement of ferrous sulfate from commercial

sources (ferric citrate will be shipped to the research pharmacy from the study sponsor). The research pharmacy will also be responsible for participant randomization and dispensation of study drug in appropriately marked containers. Written instructions on how study drugs are to be taken will be provided to all participants, and will be reinforced by face-to-face contact with study staff at the baseline and all follow-up visits.

3.6 Compliance assessment

Study drug will be provided to participants at the baseline visit (visit 1), visit 2 and visit 3. Study staff will maintain a study drug dispensing log to document drug dispending during the entire study. Participants will be instructed to return the study drug bottles including unused study drug at each follow-up visit. Any discrepancies between the remaining study drug in the bottles and the number that should be remaining will be discussed with the study participants at the time of the visits and an explanation must be documented. Participants will also be required to inform study personnel of intentional or inadvertent failure to take a study drug according to the written schedule between study visits.

3.7 Protocol for Handling of Withdrawals

3.7.1 Discontinuation of the study drug

Participants who discontinue the study drug will be expected to continue in the follow-up period and complete all study-specific visits. If a participant is unable to attend a follow-up study visit, contact by telephone is permitted to determine the reason why they are not able to attend a follow-up study visit and determine whether these assessments can be rescheduled.

The study drug can be discontinued if:

- The participant becomes pregnant
- Any investigational drug other than the study drug is used
- Any suspected drug-related adverse events or serious adverse events occur
- If any of the exclusion criteria apply during treatment (e.g., receipt of kidney transplant)
- A significant violation of the study protocol occurs.

Importantly, study drugs can be temporarily discontinued for reasons as outlined in section 3.9.1.1 below.

3.7.2 Discontinuation from the study

Participants can be withdrawn from the study at any time if they specifically ask to withdraw or if, in the opinion of the study PI, continuation of the study would be harmful to the participant. In addition, a participant can be withdrawn at any time by the PI if he or she repeatedly violates study protocol.

Participants withdrawn prior to the final scheduled visit will be asked to attend a close-out visit in which they provide blood and urine samples (for final analyses), and complete documentation related to the development of any adverse events.

If the withdrawal is due to an adverse event, participants will be followed until the event resolves or it is shown that the adverse event was not due to the study medication.

Randomized participants who withdraw prematurely will not be replaced. A participant who withdraws after signing informed consent but before randomization can be replaced.

3.8 Justification for interventions

The initial starting dose of ferrous sulfate (325 mg po tid) was chosen because this is currently the standard of care as outlined in the KDIGO guidelines for iron deficient individuals with CKD.

The starting dose of ferric citrate needed to balance the requirement to provide sufficient lowering of dietary phosphorus absorption to effect a decrease in FGF23 concentrations (and

thereby help attenuate the rise in hepcidin) while at the same time providing sufficient iron supplementation to treat iron deficiency anemia. While a starting dose of 1 gram of ferric citrate three times a day was considered, in the seminal study comparing the efficacy of ferric citrate vs. placebo in pre-dialysis CKD patients,[20] the average final dose of ferric citrate needed to achieve adequate phosphorus reduction was 5.1 grams per day. Therefore, we chose 2 grams three times per day with meals to provide sufficient phosphorus binding effect to lower FGF23 while at the same time providing adequate iron supplementation to compare against ferrous sulfate.

3.9 Procedures and Evaluations
3.9.1 Assessment of Safety
3.9.1.1 Risks and Discomforts

Phlebotomy

Subjects will experience the usual discomfort caused by phlebotomy. The total amount of blood to be collected for the entire study period will be ~150 ml, or ~ ½ cup, less than the volume of blood collected during a single blood donation (500 ml or 2 cups). We will exclude subjects with hemoglobin levels < 7 g/dl for women and < 8 g/dl for men during their screening visit.

Severe hypophosphatemia

Ferrous citrate is an oral phosphorus binder. It is possible that participants taking the prescribed ferrous citrate dose (2 grams three times a day with meals) may develop low serum phosphorus concentrations. In a prior study of 72 individuals with moderate CKD not requiring dialysis being treated with a higher cumulative dose of ferric citrate (5.1 grams/day) than proposed in the current study, there were no events of symptomatic hypophosphatemia, and only two instances of serum phosphorus concentrations falling below 2.5 mg/dL (which resolved on their own). Therefore, the likelihood of severe hypophosphatemia occurring is low, particularly since we will exclude individuals with serum phosphorus concentrations less than 3.0 mg/dL on screening labs. Nonetheless, serum phosphorus concentrations will be monitored through the study. Study drug titration will occur as follows:

- If serum phosphorus concentrations fall to between 2.5 and 3.0 mg/dL, participants will be asked to reduce the dose of ferric citrate to 1 tablet three times a day with meals.
- If serum phosphorus concentrations fall below 2.5 mg/dL, participants will be asked to hold the study drug until serum phosphorus concentrations are above 3.0 mg/dL, and then re-start the study medication at 1 tablet three times a day with meals.
- If serum phosphorus concentrations remain below 3.0 mg/dL despite reduction in study drug dose, the participant will be asked to stop ferric citrate and follow-up blood draws will be obtained two weeks after to ensure that the phosphorus concentration recover to above 3.0 mg/dL.

Elevated iron concentrations: Increases in markers of iron metabolism are anticipated in participants taking either oral ferrous sulfate or ferric citrate. In order to ensure that iron marker concentrations do not increase too high during the course of intervention, both iron saturation (TSAT) and ferritin will be monitored throughout the intervention period. If TSAT rises above 65% or ferritin rises above 1000 ng/ml at any time point the lab results will be repeated within 48 hours. If the results remain elevated, the participant will be asked to stop the study medication but will continue to be followed for the duration of the study visits.

Gastrointestinal upset: It is possible that study participants can develop gastrointestinal upset (dyspepsia, constipation, diarrhea) during the study duration. If this occurs, the following will happen:

- If the participant was randomized to the ferrous sulfate arm, he or she will be asked to reduce the dose of ferrous sulfate to twice a day. If one week after this reduction he or she continues to experience gastrointestinal upset, he or she will be asked to reduce the dose of ferrous sulfate to once a day. If one week after this reduction he or she continues to experience gastrointestinal upset, he or she will be withdrawn from the study.
- If the participant was randomized to the ferric citrate arm, they will be asked to reduce the dose of ferric citrate to 1 gram by mouth three times a day with meals. If he or she continues to experience gastrointestinal upset one week after they make this change, he or she will be withdrawn from the study.

3.9.2 Adverse events

Any adverse medical event (sign/symptom/laboratory results) occurring in a research participant will be captured in standardized forms at each follow-up study visit. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be grouped by SMQs.

A serious adverse event includes any event that results in a death, is life-threatening, results in an inpatient hospitalization, results in a significant disability, or in any other serious injury or prolongs the hospitalization as judged by the PI.

3.9.2.1 Documentation of adverse events

Each adverse event will be documented according to the following criteria:

- Intensity
    - Mild: transient in nature, minor interference in activities of daily living
    - Moderate: serious impediment to normal daily activities
    - Severe: prevents normal activities
- Relationship with study drug exposure
    - The PI will determine whether the adverse event was likely or unlikely related to study drug exposure. If the latter, the most likely alternative explanation for the adverse event will be documented in case report forms.
- Action taken
    - The study staff will document whether any action was taken to resolve the adverse event.

3.9.3 Adverse event reporting

The principal investigator will report any adverse events to the IRB within the required time frame for reporting as outlined below:

- For serious adverse events, the principal investigator will report the events to the IRB immediately (within 24 hours of event) by telephone, fax or email. The principal investigator will then submit a full written report using the IRB Adverse Event Form within 10 working days or 14 calendar days.
- For mild to moderate adverse events that are unexpected, but are thought to be related to the intervention, the principal investigator will report the events to the IRB in writing using the IRB Adverse Event Form within 20 working days or 30 calendar days.

- For mild to moderate adverse events that are both unexpected and are thought to be unrelated to the intervention, the principal investigator will summarize the events in a progress report for the annual continuing review.
- For mild to moderate and expected adverse events, the principal investigator will summarize the events in a progress report for the annual continuing review.

The principal investigator will also report all serious adverse events that occur 2 weeks after active study participation and are thought to be related to the study intervention.

4 Statistical Analyses

4.1 Primary outcome

The primary outcome variables for this analysis will be transferrin saturation (TSAT) and ferritin concentrations.

4.2 Secondary outcome variables

The secondary outcome variables will include hemoglobin, serum hepcidin, serum erythroferrone, serum phosphorus and plasma FGF23 concentrations. Serum hemoglobin, phosphorus and plasma FGF23 concentrations will be measured in all participants at all visits depending on available samples. Serum hepcidin and erythroferrone will be examined in a subset of participants (n=30) with samples available at the baseline visit, week 2 and week 6 or 12.

4.3 Statistical Plan

To compare the baseline characteristics between the two groups, student's t-test or chi square test will be used for parametric and non-parametric variables respectively. Subjects' baseline values will serve as the reference for comparison with changes post-intervention. Longitudinal changes in the primary outcome variables will be examined using repeated measures linear mixed models. In these models, time and treatment group will be the fixed variables and participants will be modeled as random terms. We will first test for a time by treatment group interaction to determine whether the change in the outcome variables over time differs by treatment group assignment. If we detect a statistically significant interaction, we will stratify the analysis by treatment group and test the main effect of time in stratified models. If we do not detect a significant interaction, we will remove the interaction term from the model and will test for the main effects of time and treatment group. If we find any differences in baseline characteristics despite randomization, we will adjust for these covariates in secondary models. We will analyze data using an intention-to-treat principle such that all randomized participants who received at least one dose of study medication and had at least one post-baseline assessment will be included to assess efficacy.

4.4 Sample Size Calculation:

The sample size estimation is powered to detect a significant difference in the change in ferritin in response to oral ferric citrate vs ferrous sulfate challenge. In a prior study, treatment with ferric citrate for 12 weeks raised serum ferritin by 77.5 [95%CI 56.2-98.7] as compared to placebo. To detect as small as a 20% lower rise in ferritin in individuals taking ferrous sulfate vs. ferric citrate over a similar period of intervention, we would need recruit 60 individuals overall (30 per arm) assuming a type I error of 0.05 and a power of 80%. This will also provide ample power to detect at least a 20% difference in the change in TSAT as well. While we also plan on examining changes in hepcidin in this protocol, we did not specifically power the analysis for this outcome variable as it is a secondary outcome.

A prior randomized, controlled study of patients with stage 3-5 CKD (eGFR 10-59 ml/min/1.73m^2) showed that treatment with oral ferrous sulfate (325 mg po bid) resulted in a mean increase in hemoglobin of 0.2 ± 0.9 g/dL (Agarwal R et al, Am J Nephrol 26:445-454, 2006). If we assume that treatment with ferric citrate for 12 weeks will increase hemoglobin by 0.6 ± 0.4 mg/dL (Block et al, Am J Kidney Dis 65(5):728-736), the current sample size of 60 participants overall will provide ~60% power to detect a mean difference in hemoglobin increase of 0.4 ± 0.7 between the two groups.

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