Bioavailability of Ferrous Sulfate Versus Ferrous Bis-glycinate in Celiac Disease

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A. Study Purpose and Rationale

Celiac disease (CD) is a chronic T-cell mediated immunologic disorder characterized by hypersensitivity to proteins found in specific grains, namely gluten, the collection of storage proteins found in wheat.[i] In response to chronic exposure to gluten and other grain proteins, both CD4 and CD8 lymphocytes accumulate in the lamina propria and epithelium of the small intestine. Tissue transglutaminase, an enzyme found in enterocytes, deamidates gluten to immunogens recognized by specific T cell populations in individuals with CD.[ii] Further supporting the immunologic etiology of CD is the fact that only individuals with certain HLA haplotypes can develop the disorder: namely, HLA-DQ2/DQ8.[iii] Although the CD4 lymphocytes in the lamina propria are those that recognize and react to the gluten fraction of the diet, equally important are the CD8 intraepithelial lymphocytes, which secrete cytokines such as interleukin-15 and alter the expression of surface antigens on enterocytes to make them more susceptible to destruction by natural killer (NK) cells.[iv] Although the details are poorly understood, this immune-cell rich milieu leads to the death of surface enterocytes, with the associated finding of villous atrophy on biopsy of CD patients. Besides physically decreasing the surface area available for absorption of nutrients, the inflammatory cytokines secreted by the T cells invading the intestinal wall also reduce absorption of macro and micronutrients, leading to the clinical picture of malabsorption, failure to thrive and steatorrhea. Formerly thought to be a rare disorder found diagnosed in childhood, in some European countries, the prevalence of celiac disease is thought to be 0.5-1%, and it is being increasingly diagnosed in adulthood.

Iron is an essential micronutrient that is absorbed preferentially in the proximal small bowel. Its absorption is linked directly to the need for iron in the body: when iron stores are decreased, iron absorption increases, and when the body is replete with iron, iron absorption decreases. The enterocyte surface transporters DMT1 (divalent metal transporter 1) and FPN1 (ferroportin 1) seem to be able to respond to these changes in iron stores by regulating iron absorption.[v] Attention has also been focused on hepcidin, a protein found on the basal surface of enterocytes that seems to regulate the response between body iron stores and intestinal iron absorption.[vi] Iron deficiency leads eventually to a decrease in red blood cell production and clinical anemia. Iron deficiency develops over months when dietary intake is inadequate; it can develop quicker in settings of continual blood loss, as body stores of iron are used to keep up with accelerated erythropoiesis.

Individuals with celiac disease have a high propensity to develop iron deficiency because of the preferential involvement of the proximal duodenum in CD, the primary site of iron absorption. In an Italian study of 1,436 patients with a primary diagnosis of gluten intolerance (i.e. celiac disease), "non-classic" symptoms predominated in 10.7% of those under 2 years of age, 28.2% of those between 2 and 14 years of age, and 61.1% of those greater than 14 years of age at the time of diagnosis (in this paper's context, "non-classic" symptomatology was defined as isolated intestinal manifestations [e.g. bloating, abdominal pain, dyspepsia] or extra-intestinal manifestations [fatigue, IDA, osteopenia, depression; dermatitis herpetiformis was considered a "classic" manifestation]). In the group consisting of children between the ages of 2 and 14, iron-deficiency anemia was found in 36.2% of the females and 21.2% of the males. In the group of individuals diagnosed with celiac disease over the age of 14, 52.4% of the females had iron-deficiency anemia, as did 12.7% of the males.[vii] Another study looked at 190 individuals diagnosed with IDA and referred for gastrointestinal work-up. Of the 190 individuals with IDA (defined in this study as hemoglobin < 14 (men), < 12 (women) and ferritin <20 ng/mL), celiac disease was ultimately diagnosed in 26 (13.7%), of whom only 11 had GI complaints. The second arm of this study looked at the resolution of iron-deficiency anemia by institution of a

gluten-free diet alone, without iron supplementation. Only 18 of the original 26 patients participated in this arm of the study. Of these 18, after six months, 14 had normal hemoglobin values but only 5 had normalized their serum ferritin values on a gluten-free diet alone. At 12 months, 17 patients had normal hemoglobin values, and 9 had normal serum ferritin. By 24 months, still only 17 had normal hemoglobin values, and 10 had normal serum ferritin. The author's conclusion was that recovery from anemia occurs with a gluten-free diet in iron-deficient CD patients, but without iron supplementation, most CD patients remain iron-deficient.[viii]

Further complicating this picture is the fact that celiac patients placed on a gluten-free diet—the only reliable means of reversing the disease's clinical manifestations—may still have subclinical disease despite strict adherence to their diet. The consequences of subclinical villous atrophy in asymptomatic celiac patients is unknown, but may continue to place them at higher risk of malabsorptive syndromes. In one series, 89 adults with celiac disease were studied over time in regard to the regression of villous atrophy on a gluten-free diet; simultaneously, their diets were consistently analyzed for trace gluten. All subjects had experienced clinical amelioration of their presenting symptoms on a gluten-free diet. Even after 8 years on a nominally gluten-free diet, 32% of the study population continued to have partial villous atrophy, and 9% had subtotal villous atrophy. After analysis of their diets, those individuals on the strictest gluten-free diets were still as likely to have villous atrophy as those individuals on a diet that permitted somewhat more gluten.[ix] The authors continue to note that the biochemical and metabolic consequences of this persistent subacute villous atrophy are unknown.

In the treatment of iron deficiency, multiple formulations of iron are available. In general, the iron salts (i.e. ferrous fumarate, ferrous sulfate and ferrous gluconate) are prescribed as iron supplements in cases of iron deficiency, and these are the most common formulations found in over-the-counter formulations as well. However, there are multiple other formulations of elemental iron available, including some that are complexed to amino acid chelators, such as FeNaEDTA or ferrous bisglycinate. Some studies have suggested that these forms of iron are more bioavailable in select populations. One such study looked at an *in vitro* model of iron absorption using human colonic adenocarcinoma cells, and found that a wheat-based cereal fortified with NaFeEDTA and ferrous bisglycinate led to increased cell ferritin formation in comparison to traditional iron salts.[x] In another study, 40 infants diagnosed with iron-deficiency anemia were treated with ferrous sulfate versus iron bisglycinate for 28 days. While both groups showed an equivalent rise in hemoglobin, the group treated with iron bisglycinate had a significantly greater increase in serum ferritin as well, suggesting better restoration of tissue iron stores.[xi] Although no study to date has looked at the differential absorption of iron formulations in celiac disease, a study looking at individuals with well-controlled Crohn's disease found that ferrous calcium citrate was better absorbed than iron bisglycinate.[xii]

Patients with celiac disease, who are often predisposed to iron deficiency, are also quite difficult to treat once they become iron-deficient. In one study looking at patients with refractory iron deficiency anemia, out of 150 patients with refractory IDA, celiac disease was diagnosed in eight, and in all of these eight, the patients were refractory to oral iron therapy.[xiii] Pareneteral iron therapy, the only alternative when oral therapy is ineffective, has many risks, including anaphylaxis, local injection site reactions and patient discomfort. Given that individuals with celiac disease, even when treated appropriately, are at greater risk for the development of IDA, and are harder to treat than non-CD patients, it would be prudent to identify oral iron regimens that are more bioavailable and tolerable in a CD population. To date, no study of this sort has been performed.

B. Study Design and Statistical Analysis

Power Calculations: With a sample size of 30 individuals, and assuming a mean Δ-iron max of approximately 16.4 in healthy controls (from Crowley et al.) with an SD of approximately 6.2, we will have 80% power with an alpha of 0.05 to detect a difference of 30% bioavailability between

the groups. Thus this sample size should provide adequate power to detect clinically significant differences in bioavailability between health controls and celiac patients if such a difference exists. Permitting 10% attrition, our target enrollment of patients is 30-35 per group.

Design: This will be a prospective, crossover, controlled clinical study to determine differences in bioavailability between an iron salt and iron-amino acid chelate in individuals with celiac disease versus controls. We hypothesize that people with celiac disease have suboptimal absorption of iron, even when their CD is well-controlled, and that they will have better absorption of an amino-acid chelated formulation as compared to conventional iron salts. We will test this hypothesis on individuals with well-controlled celiac disease treated at the Celiac Disease Center of CUMC, and controls from the general population. A subgroup analysis may be performed on a third group of individuals with poorly controlled CD as defined by persistent symptomatology, without iron deficiency.

Definitions: The bioavailability of a drug is defined as the difference in the serum AUC (area under curve) of the drug when given orally versus intravenously. In nutritional studies, the gold standard for determining the bioavailability of iron is the measurement of the incorporation of radioactive iron isotopes (Fe-55 or Fe-59) into hemoglobin two weeks after the consumption of a test meal/dose.[xiv] This is an expensive and time-consuming method. Serum iron curves, looking at the serum iron AUC after a test dose of iron, have been tested and validated as a method of estimating iron bioavailability in both dietary and pharmaceutical preparations of iron.[xv,xvi]

There are many factors that affect the bioavailability of a given drug. In the case of iron, body iron stores can affect the bioavailability of oral iron significantly. Tissue iron stores can be assessed by measuring serum ferritin, which is 100% specific in the diagnosis of iron-deficiency when a cut-off value of 15 ng/mL is used.[xvii] Serum iron is measured using a colorimetric method using ferrozine as the reagent.[xviii]

The gold standard for the diagnosis of celiac disease is a small bowel biopsy. Multiple serologic tests are available as well. The current generation of tests include the anti-endomysial IgA antibody assay, and the IgG/IgA anti-human-tissue transglutaminase antibody assay. The newer serologic tests measure the titer of anti-endomysial antibodies and anti-tissue transglutainase (EMA and tTG respectively). Their sensitivity is >90% and specificity of EMA approaches 100%.[xix] The serum anti-gp-tTG assay has an interassay CV of approximately 10% and an intraassay CV of approximately 8.7%.[xx]

Additional data that will be collected includes demographic information (i.e age, gender, race, ethnicity), anthropometric information (i.e. height, weight) and any gastrointestinal pathology reports. If small bowel biopsy was performed, the stage of celiac disease as determined by Marsh's classification (stages 1-4; based on degree of villous flattening and # of intraepithelial lymphocytes)[xxi] will be recorded.

Outcome: The primary outcome measured in this study is the bioavailability of iron for two different iron preparations in two different patient populations—those with celiac disease and those without celiac disease. Celiac disease will be defined as the presence of characteristic antibodies (either EMA or TTG) with either 1) biopsy-proven celiac disease or 2) classic symptomatology, such as GI distress. "Well-controlled" celiac disease will be defined as the subjective absence of any clinical symptoms of CD for a six month period prior to study participation while on a gluten-free diet. Objective data, such as the degree of villous atrophy on biopsy, will not be a definition of "well-controlled" celiac disease; there is literature to suggest that individuals with villous atrophy may have no GI symptoms at all, and conversely, individuals

with minimal villous atrophy may have significant GI symptoms.[xxii] Bioavailability of iron will be defined by the change in serum iron level at maximum absorption (at either 180 or 210 minutes post-dose).

Statistical Analysis: Quantitative data will be compared using a non-paired, two-tailed Student's t-test to compare bioavailability between groups. Paired t-tests will be used to compare paired data; secondary outcomes will be compared among groups by ANOVA (analysis of variance). The primary endpoints are the Δ-serum iron at 180 minutes, at 210 minutes, and the max serum iron value expressed as a percentage of the original dose. Relative iron bioavailability will be estimated by these variables; serum iron curves have been validated as an accurate reflection of iron bioavailability.[xxiii]

C. Study Procedure

Subjects will visit the Irving Clinical Research Center two times over the course of one week (7 days). Prior to first visit or at the first visit, the following information will be collected:

- Diet: Subjects will be asked to record a 3 day food diary from the day prior to the visit, which will be analyzed for iron content, sodium content, calcium content, ascorbate content, calorie/fat/protein content.
- Physical exam: All subjects will have height and weight measurements.
- Questionnaire: Age, smoking/alcohol use, past medical history, current medications/supplements.
- Serum will be collected for baseline CBC, serum iron and ferritin.

Subjects will be asked to fast from midnight on the day of the study. Subjects will arrive to the study room at 8 AM, as serum iron concentrations observe a circadian rhythm during the day. Subjects will be given the equivalent of 65 mg of elemental iron in the form of either ferrous sulfate or iron bisglycinate (see drug details for dosages). After ingestion of the test dose, 2 cc of blood will be withdrawn at 180 minutes and 210 minutes post-dose for serum iron determination. Subjects will then be told to return in exactly 7 days to have the procedure repeated with the other iron formulation. Subjects will also be asked to report any adverse side effects that they might experience from the iron dose and mail their completed questionnaires on provided stamped envelopes. Subjects will be instructed not to vary their diet, alcohol intake or take any supplemental vitamins/minerals in the interposed week between visits. At baseline on the first day, serum will be drawn for CBC, ferritin and serum iron. If these values indicate that the patient has iron deficiency at baseline (ferritin < 30 ng/mL, hemoglobin < 14 men, < 12 women), or that subjects have supranormal ferritin (>200 ng/mL), subjects will be informed and will not be asked to return for the following visit. A referral for treatment of iron deficiency will be made at the same time.

D. Study Drugs

Subjects will be given ferrous sulfate, an iron salt, and ferrous bisglycinate, an amino-acid/iron chelate.

E. Medical Devices

None.

F. Study Questionnaires

Subjects will be asked to fill out the following forms (see Appendix):

- 3 day diet recall form

- Intake history form (past medical history, medications, alcohol use, smoking history)
- Side effect profile from iron therapy

G. Study Subjects

Study subjects include all individuals between age 18-65 treated at the Celiac Disease Center at Columbia University Medical Center, as well as students/staff from CUMC for controls. For the study group, the inclusion criteria include:

- Diagnosis of celiac disease
- Institution of a gluten-free diet for *at least* six months
- During that six month period, no significant GI symptomatology, as defined as diarrhea, steatorrhea, chronic abdominal pain/discomfort/bloating.

For the control group, the only specific inclusion criteria is:

- No history of celiac disease, or a first degree family relative with the same

For the study group and control group, the exclusion criteria are as follows:

- Current iron deficiency anemia or more than one prior episode of iron deficiency anemia.
- Supranormal serum ferritin (>200 ng/mL)
- If female, pregnancy or lactation.
- Any contraindication to oral iron therapy, including hemochromatosis, peptic ulcer disease, or history of severe side effects from iron supplementation
- Inflammatory bowel disease or other malabsorptive GI diseases
- Thyroid or liver disease
- Any hematologic condition that predisposes to anemia, such as sickle cell disease, thalassemia, etc.
- Any history of chronic gynecologic bleeding (e.g. metrorrhagia), with or without a history of IDA

H. Recruitment

Patients meeting our inclusion criteria will be identified from the patient population treated at the Celiac Disease Center at CUMC, one of the largest celiac disease referral centers in the United States. Controls will be recruited from CUMC staff/students by email and flyers, and will be recruited by word-of-mouth from study participants.

I. Confidentiality

We will maintain appropriate security measures for any subject identifiers, including storing physical files in a locked cabinet to which only study staff have access and maintaining a secure database to which the PI and key study staff have access via a password.

Patients will be identified by a numerical system (e.g. patient #1 is CD#1); a master list with patient's contact information will be kept in a locked physical site so that patients with iron deficiency as measured on their first visit can be identified and contacted to make appropriate referrals. This information will be destroyed at the conclusion of our research. The only people to whom we will share PHI will be with study participants themselves if results necessitate it.

Patients will be consented as to the risks and benefits of the study and copies of all consents will be kept in a locked, secure location as well as given to the patients.

J. Conflict of Interest

None; no vitamin/supplement manufacturer is sponsoring this research.

K. Location
This is a prospective trial being conducted at Columbia University Medical Center; no other institution is participating.

L. Potential Risks
The common side effects associated with oral iron therapy include GI irritation, epigastric pain, nausea/vomiting, dark stools, stomach cramping and constipation. More rarely, patients can experience diarrhea, heartburn, or a change in urine color. Iron may also interfere with the uptake of various medications, such as penicillin or tetracycline. The risks associated with venupuncture are minor, and include bruising at the puncture site.

M. Potential Benefits
By participating, patients will be informed of their iron status, and some cases of iron deficiency may be identified. Also, by participating, celiac disease patients will be shedding light into the most appropriate way to address IDA in their population.

N. Alternative Therapies
N/A

O. Compensation to Subjects
Patients will be reimbursed travel fare if necessary, including parking at the medical center.

P. Costs to Subjects
None.

Q. Minors as Research Subjects
No minors will be participating in this study.

R. Radiation and Radioactive Substances
None.

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