Spring 5-15-2022

The Effects of Carbonyl Iron vs Ferrous Sulfate on Blood Iron Markers in Male Division III Cross-Country Athletes

Sam Gunter
University of Lynchburg, gunters843@lynchburg.edu

The Effects of Carbonyl Iron vs Ferrous Sulfate on Blood Iron Markers in Male Division III Cross-Country Athletes

Sam Gunter

Senior Honors Project

Submitted in partial fulfillment of the graduation requirements of the Westover Honors Program

Westover Honors Program

May, 2022

_____

Jill Lucas, PhD

_____

Jeffery Herrick, PhD

_____

Christine Terry, PhD

Abstract

Competitive distance runners are often predisposed to developing iron deficiency. This study aimed to determine if carbonyl iron was more effective at maintaining blood iron markers and minimizing overall fatigue and GI stress than ferrous sulfate. In this randomized, independent groups study, 7 male, division III cross-country athletes were supplemented with either carbonyl iron or ferrous sulfate for 6 weeks. Blood hemoglobin, hematocrit, subjective GI distress, subjective fatigue, and relative exertion of recent training were assessed at baseline, 3 and 6 weeks. Results were analyzed via one -way and repeated measures analyses of variance (ANOVA) to determine significant differences in outcome measures. Factorial ANOVA revealed no significant differences between hemoglobin and hematocrit levels between the two treatments. One-way ANOVA determined no significance in subjective questionnaire outcomes. Carbonyl iron was concluded to have no significantly greater effect than ferrous sulfate on the measured blood markers or subjective questions.

Key Words: Iron Supplementation, Hemoglobin, Hematocrit, Distance Runners

Project Summary

The effects from iron deficiency and resulting anemia in distance runners include excessive fatigue and feelings of weakness, resulting in impaired training and performance. Common contributing factors to these conditions include excessive blood loss, inadequate iron consumption through one's regular diet, and footstrike hemolysis (red blood cells bursting due to the repetitive foot trauma caused by the impact of running). Supplementation is often an effective way to counteract these factors. Thus, in a population such as distance runners, iron supplementation is often necessary.

While there are several kinds of iron supplements available on the market, it is often debated whether any of them are significantly more effective than others at maintaining healthy blood iron levels and overall red blood cell counts. This study is novel because it will compare two different forms of iron supplements and their effects on a competitive male distance runner population. The aim of this study was to determine if one form of oral iron supplement (carbonyl iron) is more effective than a second form (ferrous sulfate) at achieving the maintenance of iron-related blood values in a group of male distance runners. The study population used ferrous sulfate prior to testing, thus allowing ferrous sulfate to be used as a control upon beginning testing. With carbonyl iron as the experimental treatment and ferrous sulfate as the control, we hypothesized that carbonyl iron would prove more effective at maintaining the blood iron markers of hemoglobin and hematocrit. Upon statistical analysis, the carbonyl iron was not more significantly effective on blood markers or secondary, subjective outcomes than the ferrous sulfate control. Thus, the hypothesis that the carbonyl iron would prove more effective was not accepted.

Introduction

Aerobic exercise requires high amounts of oxygen delivery to working muscle cells, especially when performing activities of prolonged duration. Oxygen, when transported in the bloodstream, binds to hemoglobin, forming oxyhemoglobin. If oxygen delivery to these muscle cells is hindered, the ability to complete activity will be hindered, thus compromising performance. Iron is a critical component for hemoglobin and is required for this binding process to take place. At any given time, approximately 70 percent of iron in the body can be found binding to hemoglobin in the bloodstream or to myoglobin, which is found in muscles [1]. In the event of iron deficiency, red blood cell production will decrease; thus, the body's ability to transport and utilize oxygen will decrease as well, leading to fatigue and ultimately causing athletic performance to suffer (namely in aerobic-heavy sport athletes). Factors that commonly cause iron deficiency include inadequate diet (of iron-rich foods), menstruation (for females), gastrointestinal and genitourinary losses attributed to exercise, foot strike hemolysis (bursting of red blood cells due to the impact of running), thermohemolysis (bursting due to heat), and losses due to sweating [2]. Sweat losses of iron in particular have been shown to be significant, accounting for at least 0.4 mg after one hour of inactive sweating [3]. These factors can all lead to oxidative stress within the body, placing the athlete at further risk for increased fatigue and decreased performance [4]. Iron deficiency itself can often result in anemia, the effects of which are chronic, leading to long-term fatigue and hindered performance until iron and subsequent red blood cell production return to normal, healthy levels.

Iron deficiency is surprisingly prevalent in competitive distance runners, even for the male sex. Especially susceptible to developing this condition are younger athletes—that is, those in adolescence up through college age when development finishes—who are at increased risk for

developing any kind of iron deficiency [5]. A recent study showed that iron deficiency was prevalent in as much as 31% of sampled male competitive distance runners [6]. Distance runners require relatively large amounts of iron in their diets primarily due to the increased need for red blood cells to transport oxygen to their muscle cells. As a primarily aerobic sport, competitive distance running involves prolonged periods of high oxygen demand, placing stress on the body's ability to maintain homeostatic iron levels.

Unsurprisingly, the longer and more intense a runner's training, the greater this stress is. Marathon runners, for example, have been studied and shown to exhibit a high level of iron loss through training and competition [7]. When iron is deficient, the body's natural abilities to create hemoglobin and bind oxygen is hindered. Thus, it is key to ensure that iron levels are maintained for individuals in this population. A competitive distance runner's diet must include iron-rich foods (such as animal products, namely beef and other red meats) to ensure that levels remain healthy. Plant based options are effective alternatives, but one must consume more specific foods (dark, leafy greens for example) to maintain proper iron intake in their diet [8]. However, these foods are not always preferable by individuals with special dietary needs (whether by choice or other reasonings) who may limit heme sources of iron in their diet, so supplementation proves to be a more effective and convenient alternative for many.

Supplementation of iron can be done in various ways, the most common method being oral administration. Among this list of iron supplements, there are many different kinds, the two most popular of which are various types of iron salts (ferrous sulfate, ferrous gluconate) and carbonyl iron (which is essentially strictly elemental iron). While the two categories of synthetic supplements are often similar in total iron content, they differ in their concentrations of iron. Ferrous sulfate, arguably the most common iron salt supplement, contains about 20 percent

elemental iron. Carbonyl iron, on the other hand, is much higher in iron concentration, containing 98 percent elemental iron by weight. While iron salts are absorbed quickly by the body, carbonyl iron has a delayed absorption in the gut, beginning with its slow dissolving process in the stomach [9,10,11,12]. Although the amount of time for iron levels to increase varies, carbonyl iron has been shown to reverse iron deficiency in as little as 8 weeks [10]. While iron supplementation helps an endurance athlete maintain healthy iron levels in the body, its effectiveness must be checked by iron level testing. Endurance athletes (regardless of sex) should test their iron levels regularly, as per International Olympic Committee recommendations; once a year has been shown to be an adequate interval for routine testing, though some individuals may need to test their iron levels more often [13].

Regarding popular iron supplements, ferrous sulfate, an iron salt widely prevalent on the market, has the potential to cause gastrointestinal (GI) tract issues, with dosage not affecting the level of symptoms [14]. Carbonyl iron, however, has been shown to cause fewer GI tract issues, likely due to its slow absorption rate within the body [15]. Because it contains high concentrations of iron and low concentrations of other compounds, carbonyl iron has been shown by some studies to be absorbed very effectively by the body, and in some case it has been shown to be absorbed more effectively than its iron salt counterparts (i.e. ferrous sulfate, ferrous fumarate, etc.) [9,10,16,17,18,19,20]. Carbonyl iron has been shown by past research on different populations to be effective at combating iron-deficiency and iron-deficiency anemia, but results have been mixed based on the population studied [10,15,16,18]. The population of subjects chosen for research seems to influence whether carbonyl iron is more effective than ferrous sulfate at maintaining various blood markers like hemoglobin, hematocrit, serum iron, and serum ferritin.

In one particular study, pregnant, anemic women were supplemented with different types of iron: three different iron salts (including ferrous sulfate) and carbonyl iron [15]. The subjects in this study were randomly assigned to take one of the four supplements for their anemia. Over the course of 8 weeks, subjects took their respective iron supplement, with blood hemoglobin being tested at baseline, halfway (4 weeks in), and the conclusion of the study. The results indicated that there were no significant differences between any of the supplements, and they all had equal effects in raising the primary blood iron marker, hemoglobin. Thus, for a pregnant female population, there seems to be no significant differences in the effects of popular iron supplements.

Conversely, another study involving young children diagnosed with iron-deficiency anemia researched the two supplements for this study, carbonyl iron and ferrous sulfate, at a dosage of 5mg/kg each for a 90-day period [16]. The results indicated that carbonyl iron supplementation was more effective than ferrous sulfate at restoring blood iron markers to normal, healthy levels. While this information confirms that carbonyl iron can have a significant effect in treating iron-deficiency anemia in a young population, more research is necessary to determine its effectiveness on maintaining blood iron markers in older and more conditioned populations like aerobic sport athletes. Due to physiological differences through development—increased body size and thus increased blood volume—differences may be present. As shown with the previous study on pregnant women, hemoglobin values in particular may not differ based on choice of iron supplement. Thus, to bridge the gap in research on iron supplementation in a collegiate male athlete population, this study aims to determine if carbonyl iron is more effective at maintaining blood iron markers in a sample of male division III cross country runners.

Methods

Experimental Design

This study utilized a randomized, single-blinded model of testing, though it was not a true experiment due to the lack of a placebo for the control group. Two groups of subjects each participated in testing procedures, with 4 in the experimental group and 3 in the control group at the start of the study. Subject mortality consisted of two subjects (one from each treatment group) who left the study after visit 2 and shortly before visit 3. Both complained of either GI distress or increased fatigue levels. Thus, the study concluded with a total of 5 subjects. An independent groups design was used with each group receiving its specific treatment intervention. The first group received one tablet of a 325 mg ferrous sulfate supplement, equivalent to 65 mg of elemental iron. The second received two carbonyl iron tablets, totalling a dosage of 54 mg of elemental iron. Dietary regimens were not held constant for both groups. Upon inquiry, none of the subjects indicated being on a vegetarian diet, thus due to the sample chosen, it was deemed that all of the subjects had similar enough diets to the point that no dietary log was implemented. Blood testing was done at the start of the study (baseline), at 3 weeks (the halfway point of the study), and at 6 weeks (the end of the study). Subjects were selected on the basis of having prior experience with iron supplementation, having taken ferrous sulfate for at least one month prior to the start of the study. Thus, the first group which was administered ferrous sulfate served as a quasi-control group (though not a true one since no placebo was given), while the carbonyl iron supplement group represented the experimental group.

Subjects

Prior to the start of this study, approval was obtained from the University of Lynchburg's (Lynchburg, VA) Institutional Review Board (approval number: LHS2122023). Subjects signed both experimental informed consent and COVID-19 risk acknowledgement forms before any sort of testing. The age of the subjects ranged from 18-22 years, and all of them were males. Subjects were recruited during a practice session, during which the researchers verbally described the study that would take place. Subjects joined the study on a volunteer-associated basis and were informed of any risks and benefits associated with participation. As part of the inclusion criteria, subjects were required to have had at least one month of experience with consistent iron supplementation (of any sort, regardless of the supplement form). Subjects with no prior experience with iron supplementation (of any form) were excluded from the study. Additionally, potential subjects were disqualified from the study if they indicated significant GI distress associated with prior supplementation. All subjects were selected on the basis of currently training at the division III college level, having run consistently at least 35 miles per week for the last two months. Exceptions to this criterion were granted on the basis of short-term injuries or illness that prevented participants from running their desired mileage for a short time (less than 2 weeks). All subjects indicated that their training regimen consisted of the following: 3 days/week of easier training on Monday, Wednesday, and Thursday; 2 days/week of higher intensity training on Tuesday and Friday; 1 day per week of either prolonged aerobic conditioning or a race if subjects were competing.

Procedures

At the first testing session, subjects were given an informed consent agreement to read and sign, and they were assigned a testing number for the purposes of data confidentiality. Following this, subjects began the first session. The testing procedure utilized the Hemopoint H2 finger stick measuring system (Stanbio Laboratory, Boerne, Texas) that measured a small amount (8 microliters) of blood via microcuvette test strips (Stanbio Laboratory, Boerne, TX). Prior to blood drawing, the fingertip used for blood drawing was cleaned with an alcohol wipe. The researchers wore gloves to prevent the spread of pathogens and to protect against blood contamination. Disposable, one-time use lancets (McKesson Medical-Surgical Incorporated, Richmond, VA) were used for the drawing of blood from each participant, and each lancet was disposed of properly to eliminate any biohazards.

After each subject's finger was pricked with the lancet, gentle, upward pressure was applied on either side of the site pricked by the researcher to ensure an adequate blood sample could be gathered. Following this, a specialized microcuvette was used to extract the blood from the fingertip. These cartridges were inserted into the finger stick measuring device and used to measure blood hemoglobin and hematocrit levels. These levels were recorded securely to maintain the privacy of each subject. Subjects were provided with a bandage for the finger being used for blood sampling after their measure was taken, which was applied either by the researchers or the subjects depending upon the subject's preference. At the beginning of the second and third testing sessions, subjects were administered a subjective questionnaire which inquired about their physical state during recent training (Appendix A).

Treatments

After baseline blood testing was complete for each participant, subjects were randomly assigned based on their testing number to either of the two groups (ferrous sulfate or carbonyl iron). Subjects were then given a container of the supplements assigned to them, and they were instructed to take their supplement once daily for the course of 6 weeks. The 7 total subjects were randomly assigned to two groups, one group with 3 participants and the other one with 4. The first group received *Landau* 54 mg carbonyl iron supplement (Douglas Laboratories, Pittsburgh, PA), while the second group received *Nature Made* 325 mg ferrous sulfate supplement, equivalent to 65 mg iron (Nature Made Nutritional Products, West Hills, CA).

In the three weeks following this first blood test, subjects completed their training regimens as usual, as this study did not implement any kind of training intervention or control factor for the subjects' exercise habits. Once three weeks had passed, subjects completed their second session, which entailed a second blood test that marked the halfway point of the study, this time with the subjective questionnaires included. After another three-week period of normal training ensued, the subjects were administered one final blood test for the post-experimental measure, and also answering the subjective questionnaires upon arrival to the testing session. Prior to each blood test, subjects were instructed to maintain good hydration levels. While there was no test to measure hydration levels, subjects indicated that they were hydrated before each blood test.

Outcome Measures

Hemoglobin and hematocrit were the blood markers targeted as the primary outcome measures. Hemoglobin was measured in grams per deciliter (g/dL), while hematocrit was

measured as a percentage of blood volume that is made up by red blood cells. Each was analyzed in the Hemopoint H2 testing system. Subjective questionnaires were administered through unmarked 10-centimeter scales. For the first question regarding any new or worsening GI tract issues, subjects were asked to rate their symptoms ranging from 0 (no issues) to 10 (worst issues). For the second, subjects were asked to rate their change in fatigue level, with the left end of the scale indicating the worst negative change, the right end indicating best positive change, and the middle of the scale indicating no change at all. For the third, subjects were asked to rate any change in their overall exertion levels in daily training, and the scale was set up the same way as in the second question. Marks on the scale were then measured in centimeters to the nearest tenth and recorded.

Statistical Analysis

Statistical analysis for the hemoglobin and hematocrit blood markers was performed via factorial analysis of variance (ANOVA). Data were analyzed using the IBM SPSS statistics for Windows, version 28 (Armonk, NY). Measures for subjects at each time point were averaged based on treatment group, and a factorial ANOVA was used to determine whether time had a significant main effect on the hemoglobin and hematocrit outcome measures. Factorial ANOVA was also used to determine if there was a significant interaction between time and treatment group across the study. A 3x2 factorial ANOVA was utilized to measure the effects and interactions of time and treatment group on hemoglobin and hematocrit across all time points, respectively. A one-way ANOVA was used to determine if time had a significant main effect on subjects' answers to the subjective questionnaires. This was also used to determine if time and

treatment group had significant interactions for the answers subjects provided. Statistical significance was set *a priori* at an alpha level of 0.05.

Results

Means and standard deviations for hemoglobin across each time point for the carbonyl iron group were as follows: 13.45 ± 2.09 g/dL at baseline, 14.33 ± 1.42 g/dL at 3 weeks, and 14.63 ± 1.42 g/dL at 6 weeks (Figure 1). For the ferrous sulfate group, hemoglobin means and standard deviations were 14.20 ± 1.61 g/dL at baseline, 14.70 ± 0.72 g/dL at 3 weeks, and 14.40 ± 0.71 g/dL at 6 weeks. Hematocrit means and standard deviations for the carbonyl iron group were as follows: 40 ± 6.4% at baseline, 43 ± 4.2% at 3 weeks, and 43 ± 4.0% at 6 weeks. For the ferrous sulfate group, hematocrit means and standard deviations were 42 ± 4.7% at baseline, 42 ± 2.1% at 3 weeks, and 43 ± 2.1% at 6 weeks. Means and standard deviations for the subjective questionnaires are shown in Table 1.

With sphericity assumed, a factorial ANOVA determined that the experimental treatment of carbonyl iron had no significant main effect for time on hemoglobin ($df=2$, $F=0.401$, $p=0.686$). There was also no significant interaction between time and treatment group for hemoglobin ($df=2$, $F=0.470$, $p=0.686$). With sphericity assumed, a factorial ANOVA also determined no significant main effect for time on hematocrit across all testing points ($df=2$, $F=0.556$, $p=0.600$). This factorial ANOVA also indicated no significant interaction between time and treatment group for hematocrit ($df=2$, $F=0.401$, $p=0.686$).

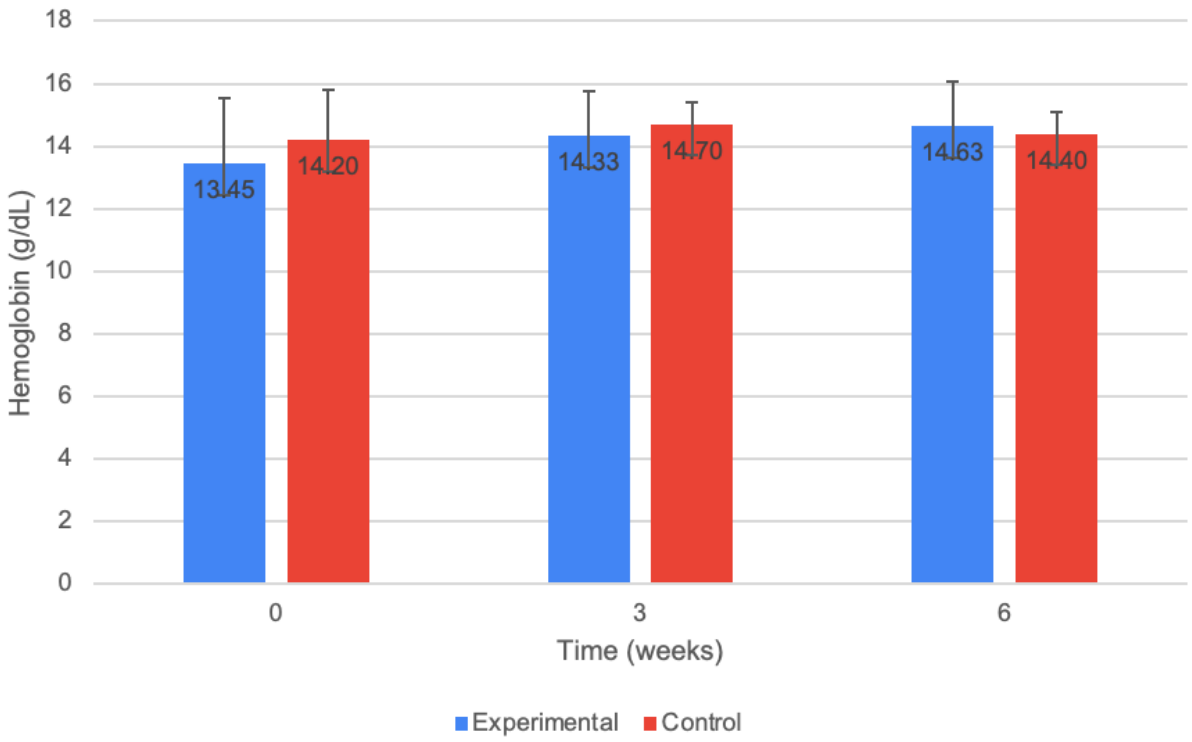

Figure 1: Hemoglobin values at 0, 3, and 6 weeks (g/dL= grams per deciliter). Experimental represents the carbonyl iron group and control represents the ferrous sulfate group. Sample sizes were 4 at baseline and 3 subjects at 6 weeks for the carbonyl iron group and 3 subjects at baseline and 2 subjects at 6 weeks for the ferrous sulfate group.

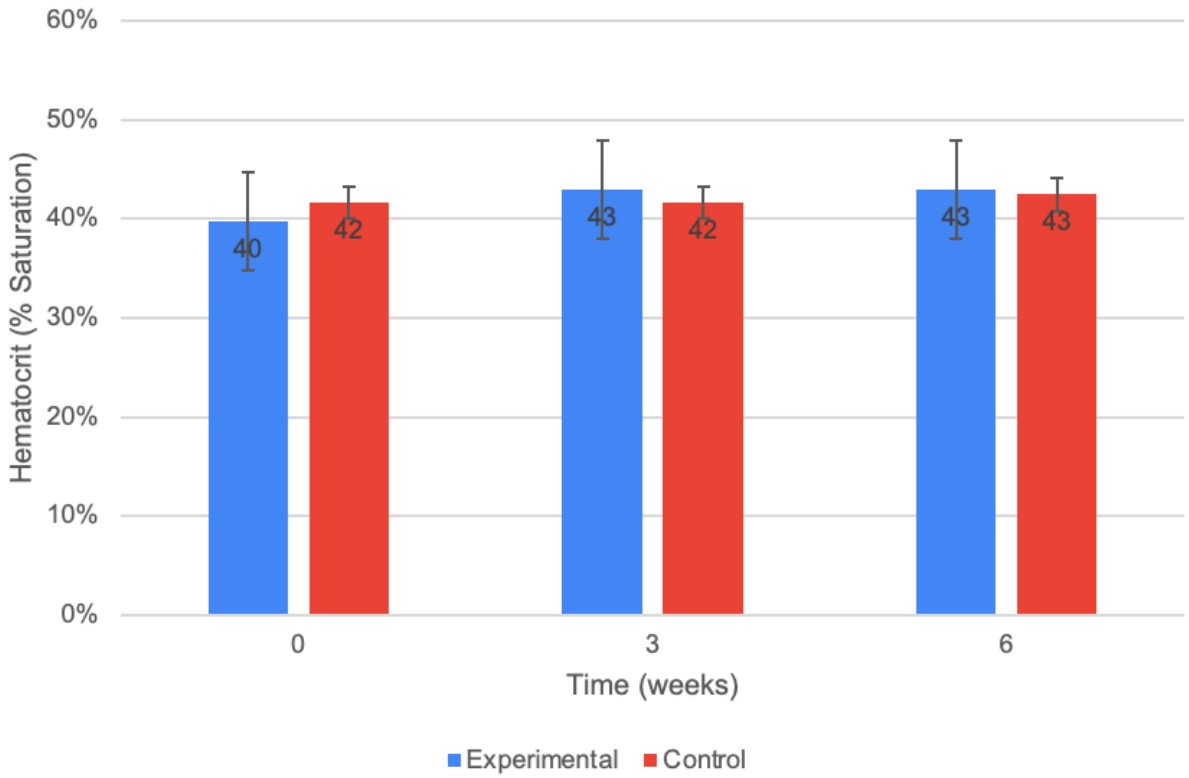

Figure 2: Hematocrit values at 0, 3, and 6 weeks(percent saturation). Experimental represents the carbonyl iron group and control represents the ferrous sulfate group. Sample sizes were 4 subjects at baseline and 3 subjects at 6 weeks for the carbonyl iron group and 3 subjects at baseline and 2 subjects at 6 weeks for the ferrous sulfate group.

As for the subjective questionnaires, a one-way ANOVA determined no statistical significance in any of the three questions at the second and third testing sessions during which questionnaires were utilized. Means and standard deviations are shown in Table 1. For GI tract issues at each session, values closer to zero indicate few reported adverse effects. For fatigue and exertion, values closer to 5 indicate less change. The higher the value is above 5, the greater the positive change, and the more it is below 5, the greater the negative change.

Each question was compared between time points, and this one-way ANOVA was used to determine if time had a main effect and if time and treatment had a significant interaction for each point. There was no significant main effect for time on GI tract issue ratings between 3 and 6 weeks ($df=1$, $F=1.029$, $p=0.385$). There was also no significant main effect for time on fatigue or exertion ratings between 3 and 6 weeks ($df=1$, $F=0.411$, $p=0.567$; $df=1$, $F=0.581$, $p=0.501$; respectively). There was no significant interaction between time and treatment group for question GI tract issues, fatigue, or exertion ($df=1$, $F=0.257$, $p=0.647$; $df=1$, $F=0.036$, $p=0.862$; $df=1$, $F=0.000$, $p=0.985$; respectively).

Table 1: Mean and standard deviation values for each subjective question asked to the subjects.

		CI (mean ± SD)	**FS** (mean ± SD)
GI Issues	Week 3	2.43 ± 4.05	0.00 ± 0.00
	Week 6	0.73 ± 1.27	1.10 ± 0.14
Exertion	Week 3	5.65 ± 0.97	5.20 ± 0.96
	Week 6	5.57 ± 0.49	4.85 ± 0.07
Fatigue	Week 3	5.20 ± 0.96	4.23 ± 2.11
	Week 6	4.85 ± 0.07	4.80 ± 0.14

(For question 1, a "0" was used to indicate no change, whereas for questions 2 and 3, a "5" was used to indicate no change, as this was the center of the scale.) Carbonyl iron group means and standard deviations are listed as "CI," whereas ferrous sulfate group means and standard deviations are listed as "FS."

Discussion

The main finding of this study was that carbonyl iron exhibits no significant differences from ferrous sulfate as measured by the blood markers of hemoglobin and hematocrit when all subjects were previously taking ferrous sulfate supplements. Additionally, subjects did not indicate any significant improvements or differences in GI distress, overall fatigue, or exertion in daily training from taking carbonyl iron as opposed to ferrous sulfate. While the carbonyl iron treatment did not demonstrate itself to be any more effective in maintaining/increasing the measured blood iron markers, it did prove equally effective in doing so, as demonstrated in the results. This lack of significant differences suggested that carbonyl iron supplementation is similar to ferrous sulfate supplementation for a male collegiate distance runner population, though more research is needed to investigate this.

These findings were inconsistent with most previous research on different subjects where carbonyl iron has been shown to prevent and resolve iron deficiency more effectively than iron salts like ferrous sulfate in some populations [10,16]. Farias et al. demonstrated that carbonyl iron exhibits a greater capacity to cure iron deficiency anemia in children, as evidenced through a greater variety of blood markers measured [16]. For example, in their study, hemoglobin demonstrated an increase of 1.3 g/dL for subjects on carbonyl iron and an increase of 1.2 g/dL for those on ferrous sulfate over the course of 30 days [16]. Additionally, hematocrit increased by 4.1 percent for the carbonyl iron group, while ferrous sulfate only increased by 3.5 percent for the same time period. After 90 days, the change in hematocrit levels was even more significant for the carbonyl iron group, increasing by 5.3 percent from baseline as opposed to the ferrous sulfate group's increase of only 4% from baseline. The study also measured serum ferritin, a common indicator of healthy versus unhealthy iron stores within the body [16]. The wider array

of variables may have allowed them to determine with greater specificity the level of significance in differences between carbonyl iron and ferrous sulfate. Additionally, their subjects were young and suffered from iron-deficiency anemia, differing drastically from the college-aged subjects from the present study with already healthy iron levels—that is, the subjects of this current study did not present any signs or symptoms of anemia. The differences in subjects' initial iron status may have accounted for most of the discrepancies in results between the two studies. Additionally, subject age and factors influenced by age may have played a part in these differences.

Conversely, Gamad et al. found no significant differences in supplementing with carbonyl iron versus ferrous sulfate [15]. As was the case in this present study, they primarily measured various forms of hemoglobin [15]. However, Gamad et al. studied pregnant women diagnosed with iron-deficiency anemia and found that carbonyl iron was no more effective at reversing the subjects' anemia than ferrous sulfate or any of the other iron salts studied [15]. With hemoglobin as the primary outcome variable, no significant differences were observed between the levels of change in the carbonyl iron group or the ferrous sulfate group from baseline until the conclusion of the study at 8 weeks (9.7 ± 0.75 to 11 ± 0.93, and 9.6 ± 0.73 to 11 ± 1.14, respectively). These findings were similar to the findings of this study at present in that carbonyl iron may not have a significantly greater enhancing effect on hemoglobin compared to ferrous sulfate.

Using subjective questionnaires to measure adverse effects are not commonly measured reports, but some research has suggested potential significance in subjects' toleration of a carbonyl iron supplement [15,10]. In Gamad et al., subjects experienced higher levels of adverse effects, namely GI tract issues, when taking ferrous fumarate as opposed to carbonyl iron, suggesting that a form of iron absorbed more slowly may be consumed with less GI disturbance

than an iron salt [15]. The subjects from Marks et al. indicated high toleration for carbonyl iron, as many kept taking the supplement on their own after the conclusion of the study, suggesting low levels of GI disturbance [10]. Past research has shown that the overall adverse incidence rate for ferrous sulfate is 32.3%, though no conclusive percentages have been shown for carbonyl iron [14]. In the present study, however, there were no significant differences in the subjective responses of GI distress, overall fatigue, and levels of relative exertion to each supplement. This was likely due to the fact that the subjects were already taking a ferrous sulfate supplement and were not experiencing any significant GI distress, and thus introducing a new form of iron which has demonstrated itself to sometimes be less harmful to the GI tract would not affect them any better or worse [10,15,17**,**18**,**19,20].

The reasoning for these results is likely due to several factors. For one, subjects were already taking iron in the form of ferrous sulfate (which then served as the quasi-control) prior to the start of experimentation. Thus, iron was likely already present in healthy amounts in their bodies, as evidenced by their healthy levels of hemoglobin and hematocrit at baseline, so it would have been unreasonable for them to exhibit a high degree of change. While some previous studies have indicated that carbonyl iron can provide a better and more easily tolerated absorption compared to other forms of the mineral, the amounts of iron in each supplement used for this study differed, thus serving as a limitation [10**,**16**,**20]. In the control group, 325 mg of ferrous sulfate was administered to subjects, equivalent to 65 mg of iron. The carbonyl iron supplement, on the other hand, while 98 percent elemental iron by weight (as opposed to ferrous sulfate which is only 20 percent elemental iron by weight), was only available in amounts of up to 54 mg for serving size. This difference may have contributed to the results shown in the study.

Previous research has used serum ferritin as a blood marker for indicating iron storage levels within the body [10,16]. While serum ferritin is a commonly measured blood iron marker—due to its low fluctuation levels and its high correlation with iron deficiency—the study at present did not assess it due to the prohibitive cost of the tools needed to measure it. Because of this, the present study was only able to measure hemoglobin and hematocrit due to available resources. Hemoglobin and hematocrit were chosen as primary outcome variables due to their relevance in indicating blood iron status, as previous studies have shown that these variables are important in showing differences in blood marker values [10,16]. However, serum ferritin is a more stable variable—that is, not greatly affected by hydration status—making it a more consistent indicator of blood iron status.

Diet could have been a potential source of small variance that was not controlled for in this study, as certain individuals may have consumed more iron rich foods in a given day than others. (It is important to note that while drastic variance in diet was unlikely due to all subjects dining from the same food provider, some variance in day-to-day diets was possible.) Though none of the participants indicated that they followed a vegetarian or vegan diet, it was possible that some subjects regularly consumed more iron-rich foods than others. However, due to the limited scope of the participant sample and the dietary indications of the participants, dietary logs were not kept. Subjects ate regularly at the various dining services provided by the University of Lynchburg. Thus, their food supply was considered similar enough to rule out significant dietary differences from person to person. Additionally, subjects were encouraged to take their respective supplement at the same time each day, and to do so apart from any type of calcium-rich food, which can interfere with iron absorption. Subjects were also instructed to stay regularly hydrated, especially on testing days. These factors helped to regulate both the

absorption of the iron itself and the consistency of the outcome measures which indicated how well the iron was absorbed. Nevertheless, small variations in diet could have contributed to the results of this study.

On the contrary, it was not known whether subjects took any sort of vitamin C supplement or any other supplement which could increase iron absorption or overall intake of the mineral. These factors were not directly controlled, as the present study did not seek to mitigate the usage of other supplements besides iron that subjects may have regularly taken. In addition to extraneous supplemental factors that could affect iron levels, there was also the variable of training intensity/volume fluctuation which could not be controlled for. Though the extent of subjects' running was required to be a minimum of 35 miles per week consistently, the intensity and volume beyond that (and thus subsequent fatigue levels) were subject to change. However, these fluctuations in training load for all the subjects—if it changed at any point during the study—would have changed equally for each subject, thus negating any unnecessarily large discrepancies in fatigue levels between participants. Nevertheless, these fluctuations in training load may have played a role in affecting hemoglobin and hematocrit levels, and they likely played a larger role in the responses to the subjective questionnaires. During weeks of training with higher volume or intensity, subjects may have been more likely to report higher levels of fatigue and exertion in their training, thus affecting their responses to the subjective questionnaires. It was unable to be determined when subjects were on higher versus lower volume/intensity bouts of training.

Potential changes to be implemented in future studies could include a more detailed blood panel, one that encompasses variables like serum iron, total iron binding capacity, and serum ferritin in addition to hemoglobin and hematocrit. This would allow for more precise

measurements of the effects of the two iron supplements, as one variable may show significantly greater change than another. Additionally, a larger sample size would promote greater generalizability; this study only involved a small sample of competitive distance runners due to the availability of nearby subjects who met the inclusion criteria. For future studies on this population, it would be necessary to include a more detailed blood panel and also to control the diets and hydration levels of all subjects (including the levels of iron in their respective supplements) to ensure equal levels of iron consumption.

The findings of this study clearly indicated no significant differences in main effects for time on hemoglobin and hematocrit. The interactions between time and treatment groups for hemoglobin and hematocrit were also not statistically significant. Additionally, the secondary outcomes—those being the subjective questionnaires—indicated no significant differences between carbonyl iron and ferrous sulfate on GI distress, fatigue, or exertion levels. Thus, in seeking to discover if carbonyl iron was more effective for maintaining blood iron markers, this study demonstrated that it was not. None of the subjects in the experimental group indicated that their supplement was more or less effective, whether that was demonstrated through the objective blood marker numbers or their subjective answers to questionnaires. Thus, the hypothesis that carbonyl iron would be a more effective iron supplement on the measured blood markers was not accepted.

Practically, this study indicates that a population of competitive male distance runners can choose either carbonyl iron or ferrous sulfate (or likely another iron salt) to supplement with, as their effects have been shown to be similar. For individuals in this group with already healthy levels of hemoglobin and hematocrit, the type of iron supplement chosen does not seem to be a significant factor in mitigating losses of iron and/or further improving iron status. While GI

discomfort is a common complaint amongst individuals supplementing with iron, neither carbonyl iron nor ferrous sulfate demonstrated itself to cause more GI tract issues, suggesting that a competitive male distance runner population could benefit from taking either. Additionally, due to this lack of significant differences between the two iron supplements, it is likely that one may switch freely between the two supplements without having to expect any significant adverse effects or changes in fatigue from training. Though some may hold to the idea that certain forms of iron supplementation are superior (or inferior) to others for male distance runners, this study has shown two very different types—carbonyl iron and ferrous sulfate—to behave very similarly in subjects who have already supplemented with ferrous sulfate, thus defying that notion. Therefore, though some may experience individual differences that cause adverse effects, a competitive male distance runner population should fare equally well when supplementing with carbonyl iron as it would with ferrous sulfate.

References

1. Hemoglobin and Functions of Iron [Internet]. cited August 25, 2021. Available from: https://www.ucsfhealth.org/education/hemoglobin-and-functions-of-iron

2. Suedekum NA, Dimeff RJ. Iron and the athlete. Curr Sports Med Rep. 2005 Aug;4(4):199-202. doi: 10.1097/01.csmr.0000306207.79809.7f. PMID: 16004828.

3. Vellar OD. Studies on sweat losses of nutrients I. iron content of whole body sweat and its association with other sweat constituents, serum iron levels, hematological indices, body surface area, and sweat rate. Scand J Clin Lab Invest [Internet]. 1968;21(2):157-67. Available from: https://doi.org/10.3109/00365516809084278

4. Imai N, Kobayashi Y, Uenishi K. The intake of kiwifruits improve the potential antioxidant capacity in male middle- and long-distance runners routinely exposed to oxidative stress in Japan. Sports (Basel); Sports (Basel) [Internet]. 2021;9(3):37.

5. Koehler, K., Braun, H., Achtzehn, S. *et al.* Iron status in elite young athletes: gender-dependent influences of diet and exercise. *Eur J Appl Physiol* 112, 513–523 (2012). https://doi.org/10.1007/s00421-011-2002-4

6. Coates A, Mountjoy M, Burr J. Incidence of iron deficiency and iron deficient anemia in elite runners and triathletes. Clin J Sport Med [Internet]. 2017;27(5):493-8.

7. Zourdos MC, Sanchez-Gonzalez M, Mahoney SE. A brief review: The implications of iron supplementation for marathon runners on health and performance. Journal of strength and conditioning research; J Strength Cond Res [Internet]. 2015;29(2):559-65.

8. Nebl J, Schuchardt JP, Ströhle A, Wasserfurth P, Haufe S, Eigendorf J, Tegtbur U, Hahn A. Micronutrient status of recreational runners with vegetarian or non-vegetarian dietary patterns. Nutrients; Nutrients [Internet]. 2019;11(5):1146.

9. Bianco C, Brittenham G, Gilcher R, Gordeuk V, Kushner J, Sayers M, Chambers L, Counts R, Aylesworth C, Nemo G, Alving B. Maintaining iron balance in women blood donors of childbearing age: Summary of a workshop. Transfusion [Internet]. 2002;42(6):798-805.

10. Marks DC, Speedy J, Robinson KL, Brama T, Capper HR, Mondy P, Keller AJ. An 8-week course of 45 mg of carbonyl iron daily reduces iron deficiency in female whole blood donors aged 18 to 45 years: Results of a prospective randomized controlled trial. Transfusion [Internet]. 2014;54(3):780-8.

11. Manoguerra AS, Erdman AR, Booze LL, Christianson G, Wax PM, Scharman EJ, Woolf AD, Chyka PA, Keyes DC, Olson KR, Caravati EM, Troutman WG. Iron ingestion: An evidence-based consensus guideline for out-of-hospital management. Clinical toxicology (Philadelphia, Pa.); Clin Toxicol (Phila) [Internet]. 2005;43(6):553-70.

12. Nagpal J, Choudhury P. Iron formulations in pediatric practice. Indian pediatrics [Internet]. 2004;41(8):807-15. Available from: https://www.ncbi.nlm.nih.gov/pubmed/15347868

13. Parks RB, Hetzel SJ, Brooks MA. Iron deficiency and anemia among collegiate athletes: A retrospective chart review. Med Sci Sports Exerc [Internet]. 2017;49(8):1711-5.

14. Tolkien Z, Stecher L, Mander AP, Pereira DIA, Powell JJ. Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: A systematic review and meta-analysis. PloS one; PLoS One [Internet]. 2015;10(2):e0117383.

15. Gamad N, Saha PK, Sharma P, Suri V, Chakrabarti A, Saha L. A randomized controlled trial comparing the efficacy, tolerability, and cost of oral iron preparations in iron‐deficiency anemia in pregnancy. J Obstet Gynaecol Res [Internet]. 2021;47(11):3828-41.

16. Farias ILG, Colpo E, Botton SR, Silveira RB, Fleig A, Schimitz CAA, Silva JEP. Carbonyl iron reduces anemia and improves effectiveness of treatment in under six-year-old children. Revista brasileira de hematologia e hemoterapia [Internet]. 2009;31(3):125-31. Available from: https://explore.openaire.eu/search/publication?articleId=od_____3056::c54fbf81346f3362a85330784e0cb8d3

17. Manoguerra AS, Erdman AR, Booze LL, Christianson G, Wax PM, Scharman EJ, Woolf AD, Chyka PA, Keyes DC, Olson KR, Caravati EM, Troutman WG. Iron ingestion: An evidence-based consensus guideline for out-of-hospital management. Clinical toxicology (Philadelphia, Pa.); Clin Toxicol (Phila) [Internet]. 2005;43(6):553-70.

18. Nagpal J, Choudhury P. Iron formulations in pediatric practice. Indian pediatrics [Internet]. 2004;41(8):807-15. Available from: https://www.ncbi.nlm.nih.gov/pubmed/15347868

19. Gordeuk VR, Brittenham GM, McLaren CE, Hughes MA, Keating LJ. Carbonyl iron therapy for iron deficiency anemia. Blood. 1986 Mar;67(3):745-52. PMID: 3947745.

20. Adsul BB, Desai A, Gawde A, Baliga V. Comparative assessment of the bioavailability, efficacy and safety of a modified-release (MR) carbonyl iron tablet and oral conventional iron preparation in adult Indian patients with nutritional iron deficiency anemia. J Indian Med Assoc. 2005 Jun;103(6):338-42. PMID: 16225161.

Appendix A: Questions administered to subjects at 3 weeks and 6 weeks

On a scale of none at all to the worst possible symptoms, how bad have your side effects (*overall side effects, level of GI tract issues experienced, headaches)* been, if any, while taking your iron

supplement?

No Symptoms _____Worst Symptoms

On a scale of negative physical change to positive physical change, how has your overall exertion in each training session changed compared to pre-testing (i.e. does it feel any harder to get through each training run)? (NOTE: If no change has occurred, please mark the middle of the scale.)

Negative Change _____Positive Change

On a scale of negative physical change to positive physical change, how has your overall fatigue experienced from training changed compared to pre-testing? (NOTE: If no change has occurred, please mark the middle of the scale.)

Negative Change _____Positive Change