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ORIGINAL CONTRIBUTIONS

Comparison of Oral Iron Supplement Formulations for Normalization of Iron Status Following Roux-EN-y Gastric Bypass Surgery: a Randomized Trial

Renee A. Mischler[1] · Seth M. Armah[2] · Bruce A. Craig[3] · Arthur D. Rosen[4] · Ambar Banerjee[5] · Don J. Selzer[5] · Jennifer N. Choi[5] · Nana Gletsu-Miller[1]

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Abstract

Background The evidence behind recommendations for treatment of iron deficiency (ID) following roux-en-y gastric bypass surgery (RYGB) lacks high quality studies.

Setting Academic, United States

Objective The objective of the study is to compare the effectiveness of oral iron supplementation using non-heme versus heme iron for treatment of iron deficiency in RYGB patients.

Methods In a randomized, single-blind study, women post-RYGB and iron deficient received non-heme iron ($FeSO_4$, 195 mg/day) or heme iron (heme-iron-polypeptide, HIP, 31.5 to 94.5 mg/day) for 8 weeks. Measures of iron status, including blood concentrations of ferritin, soluble transferrin receptor (sTfR), and hemoglobin, were assessed.

Results At baseline, the mean ± standard deviation for age, BMI, and years since surgery of the sample was 41.5 ± 6.8 years, 34.4 ± 5.9 kg/m^2, and 6.9 ± 3.1 years, respectively; and there were no differences between $FeSO_4$ (N = 6) or HIP (N = 8) groups. Compliance was greater than 94%. The study was stopped early due to statistical and clinical differences between groups. Values before and after $FeSO_4$ supplementation, expressed as least square means (95% CI) were hemoglobin, 10.8 (9.8, 11.9) to 13.0 (11.9, 14.0) g/dL; sTfR, 2111 (1556, 2864) to 1270 (934, 1737) μg/L; ferritin, 4.9 (3.4, 7.2) to 15.5 (10.6, 22.6) μg/L; and sTfR:ferritin ratio, 542 (273, 1086) to 103 (51, 204); all $p < 0.0001$. With HIP supplementation, no change was observed in any of the iron status biomarkers (all $p > 0.05$).

Conclusions In accordance with recommendations, oral supplementation using $FeSO_4$, but not HIP, was efficacious for treatment of iron deficiency after RYGB.

Keywords Iron deficiency · Iron supplementation · Nutritional complications · Bariatric surgery

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✉ Nana Gletsu-Miller
ngletsum@purdue.edu

[1] Department of Nutrition Science, Purdue University, 700 W. State Street, Stone Hall, Room 208, West Lafayette, IN 47907, USA

[2] Department of Nutrition, University of North Carolina at Greensboro, Greensboro, NC 27412, USA

[3] Department of Statistics, Purdue University, West Lafayette, IN 47907, USA

[4] Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA

[5] School of Medicine, Indiana University, Indianapolis, IN 46202, USA

Abbreviations

RYGB	Roux-en-y gastric bypass
BMI	Body mass index
CRP	C-reactive protein
sTfR	Soluble transferrin receptor
TIBC	Total iron binding capacity
HIP	Heme iron polypeptide

Introduction

Bariatric surgery to treat severe obesity is common in the USA today. About 1.5 million people have undergone bariatric surgery already, and an additional 200,000 individuals undergo the surgery every year [1]. Roux-en-Y gastric bypass (RYGB) is currently one of the most popular types of bariatric surgery procedures, comprising 34.2% of all bariatric procedures [2]. RYGB promotes significant weight loss through both energy

restriction (by reducing the size of the stomach, which increases satiety [3–5]) and macronutrient malabsorption (by bypassing part of the intestine which is needed for adequate nutrient absorption [6]). For moderately and severely obese individuals, this surgery is effective for weight loss, often after they have failed using conventional dieting, and it can reduce the risk of type 2 diabetes, cancer, heart attack, stroke, and other obesity-related comorbidities [7, 8].

Unfortunately, surgically-induced deficiencies in essential nutrients are prevalent after bariatric surgery, and iron deficiency occurs in 10 to 60% of patients after surgery [9–12]. Iron plays a critical role in the body, and this sheds light into the signs and symptoms that manifest when an individual is iron deficient. The clinical consequences of iron deficiency in bariatric surgery patients have been shown to negatively impact quality of life [13] and include general fatigue, weakness, cold intolerance, anemia, hair loss, pica, and cognitive impairments in concentration, attention, and memory [14–17]. Because of issues such as a lack of monitoring and screening, reduced dietary intake of bioavailable iron, poor compliance to prophylactic iron supplementation, and poor response to treatment, bariatric surgery patients who develop iron deficiency often remain undiagnosed and untreated [1]. Nutritional management of iron status needs to be improved for patients to reap the full benefits of bariatric surgery.

The standard of care recommendation for treatment of iron deficiency after bariatric surgery, as stated in the clinical practice guidelines, is to provide up to 150–200 mg of oral non-heme iron daily [18, 19], commonly in the form of ferrous sulfate, ferrous fumarate, or ferrous gluconate. The evidence that supports these recommendations is not ideal since it is based on a randomized control trial that evaluated oral ferrous sulfate when used as a prophylactic [20] as opposed to for treatment of overt iron deficiency. Moreover, in this trial, and in another that did not have a comparator group [21], oral iron supplementation did not improve anemia. Oral iron supplementation was also found to be ineffective for resolution of iron deficiency in observational studies [17, 22–24]. Since iron deficiency after bariatric surgery appears to be refractory to treatment with oral iron supplementation, current guidelines may need to be revisited.

The ineffectiveness of oral iron supplementation for prevention or treatment of iron deficiency may be due to reduced intestinal absorption of iron [25, 26] or to low compliance of patients to the regimen [27, 28]. Users of ferrous sulfate and other formulations of non-heme iron preparations experience gastrointestinal adverse side effects such as diarrhea and constipation because inorganic iron is highly labile [29]. However, in a recent cross-sectional study, we demonstrated that prophylactic use of oral non-heme iron supplementation was adequate for preserving normal iron status following RYGB [30]. In addition, we demonstrated that high dietary intake of heme iron was also associated with positive iron status after bariatric surgery [30]. In addition, studies assessing intestinal iron absorption in severely obese patients, using the stable isotope method which is highly accurate, found that absorption of heme iron was twofold higher compared to non-heme iron [25]. For this reason, we speculated that oral supplementation with an iron formulation based on heme iron may be better when compared to non-heme iron formulations for treatment of iron deficiency post bariatric surgery.

Heme iron polypeptide (HIP) is a novel supplemental formulation of heme iron that exists commercially, under the brand name Proferrin ES (Colorado Biolabs Inc., Frederick, CO). In healthy subjects, the bioavailability of HIP was greater than that for ferrous sulfate [31], but HIP's effectiveness was comparable to ferrous sulfate when used as a treatment for iron deficiency in patients with chronic kidney disease [32]. HIP has not been evaluated in patients who have had bariatric surgery. Therefore, the objective of this study was to compare the effectiveness of oral supplementation using HIP versus ferrous sulfate for treatment of iron deficiency in a bariatric surgery population. Since ferrous sulfate is the standard of care, we did not include a placebo group, which would be unethical. To our knowledge, this is the first randomized clinical trial to provide empirical information regarding oral iron formulations for treatment of iron deficiency in bariatric surgery patients, which can be used to inform clinical practice guidelines. The study is registered at ClinicalTrials.gov (NCT 02404012).

Methods

Participant Recruitment and Eligibility

Participants who already had a bariatric surgery procedure, at least 6 months ago, were recruited through the use of a practice-based research database within Indiana Network of Patient Care at Regenstrief Institute which is offered through the Research Recruitment Office at the Indiana Clinical and Translational Science Institute. The investigators received approval from the Institutional Review Board of Purdue University to conduct the study (#1410015305). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

A screening visit was used to identify individuals who were eligible for the study. Participants visited the Indiana Clinical and Translational Science Institute at Purdue University (West Lafayette) or at Indiana University Purdue University at Indianapolis. Eligibility criteria was reviewed and interpreted by a study physician. Participants were eligible if they were

women aged 18 to 50 years, who had undergone RYGB at least 6 months previously. Since we were seeking to enroll RYGB patients who were iron deficient, we expected that both recent and patients further out from surgery would be eligible for the study. Women who were under 50 years of age, but were post- or peri-menopausal (e.g., due to hysterectomy), were included in the study. Individuals were excluded if they were pregnant, had a surgical revision of a bariatric procedure, had intravenous (IV) iron greater than 1 month previously, had been recently hospitalized for an acute illness, or were not classified as iron deficient. A single, reliable biochemical indicator that accurately diagnoses iron deficiency does not exist; for this reason, using multiple parameters provides the best assessment of iron status [33]. Patients were classified as iron deficient if they met at least two of the following conditions at their screening visit: (1) ferritin < 20 μg/L [33], (2) total-iron binding capacity (TIBC) > 370 μg/dL [33], (3) soluble transferrin receptor (sTfR) > 2012 μg/L, and (4) sTfR:ferritin ratio > 500 [33].

Clinical Biochemistries

After participant had undergone an overnight fast of at least 8 h, a blood draw was obtained using venipuncture, and blood was collected for analysis of iron status (ferritin, hemoglobin, TIBC, and sTfR), as well as copper, zinc, C-reactive protein (CRP), plasma hepcidin, and vitamins B_6, B_9, and B_{12}. With the exception of plasma hepcidin which was measured using ELISA kit (DRG International, Springfield, NJ, USA), all other laboratory assays were undertaken by Mid America Clinical Laboratories (MACL, Indianapolis, IN, USA) which is a commercial reference clinical laboratory. At the end of the screening visit, to ensure that other micronutrient deficiencies, besides iron, were prevented or treated, participants were provided with a chewable (iron-free) multivitamin/mineral supplement (Centrum Silver, Pfizer Inc., Kings Mountain, NC, USA) and instructed to take the multivitamin once daily. The multivitamin provided the following nutrients: 75 mg vitamin C, 2.2 mg vitamin B1, 7 mg vitamin B_6, 25 mcg vitamin B_{12}, 200 mg calcium, 15 mg zinc, and 2 mg copper.

Description of Intervention

The study used a randomized, controlled design, in which participants were randomized to receive either the standard of care, ferrous sulfate, or HIP for 8 weeks, with follow-up visits scheduled at 2, 4, and 8 weeks. We initially considered testing similar dosages of iron in the two formulations; however, heme iron is more bioavailable than non-heme iron, 25 versus 10% absorption, respectively [34]. In addition, we were informed by our previous study, which found that heme iron, when found in food, is a stronger predictor of iron status compared to non-heme iron [30]. Therefore, we compared the dose of non-heme iron that is recommended for bariatric surgery patients (195 mg non-heme iron/day) [18], to HIP at a dose of 31.5 mg heme iron/day, which is the dose recommended by the manufacturer (Colorado Biolabs Inc., Frederick, CO), and 1.5 times the dose that was used in an absorption study with healthy subjects [31]. Based on an interim analysis, specified in the initial protocol, and conducted after eight patients were enrolled in the study, the Data Safety Monitoring Committee advised including higher doses of HIP (containing 63 mg iron/day or 94.5 mg iron/day). The two additional doses were included in the randomization as a 1:1:1:1 allocation ratio. A web-based site called Stat Trek Random Number Generator was used to generate the random allocation sequence. Throughout the study, participants were blinded to their assignment group. To meet the dose of iron administered, patients were instructed to take one pill of HIP or ferrous sulfate three times per day. In addition to the iron supplement, participants were told to continue taking the same daily iron-free multivitamin/mineral supplement as they were instructed to take at their screening visit. Based on previous findings from our laboratory, we found that ingestion of iron and vitamin C from food and supplements, predicted iron status, but other nutrients, including calcium, did not [30]. Therefore, we did not provide specific instructions about the mode or timing of ingestion; we only asked participants to record, in a medication diary, when they took a supplement and whether they took it with food.

Quality of Formulations

To ascertain that the amount of iron in the two different supplements was equivalent to that reported by the manufacturers, samples of these supplements were analyzed for their iron content using inductively-coupled plasma mass spectrometry [35]. For both supplements, the amount of iron that was measured was comparable to that reported by the manufacturer (for ferrous sulfate, 67.2 mg iron/pill measured versus 65.0 mg/pill reported; for HIP, 11.8 mg iron/pill measured versus 10.5 mg reported by manufacturer). A simple disintegration test was conducted according to a protocol advised by the ConsumerLab®, a commercial company that analyzes the quality of dietary supplements [36]. The solubility of $FeSO_4$ and HIP pills was tested by stirring one pill in 100 mL of water at 37 °C, for 30 min. At the end of the test, which was done in triplicate, none of the $FeSO_4$ supplement remained; whereas, 44% of the HIP pill remained intact.

Assessment of Nutrient Intake

Before each clinic visit (at baseline, 2-, 4-, and 8-week follow-up), participants were given a 3-day food/supplement log to complete beforehand and turn in during the visits. Participants were instructed to complete the logs on 3 non-consecutive

days (2 weekdays and 1 weekend day) prior to their visit. Participants recorded whether they crushed the pills or took them whole, and whether they took them with or without food. During the study visits, the study registered dietitian reviewed the log for accuracy, completeness, portion sizes, and cooking methods, and to clarify any uncertain food items. The food records were analyzed using Nutrition Data System for Research software, version 2013 (University of Minnesota, Minneapolis, MN, USA), and average daily intake of nutrients was calculated. Because the dietary analysis software did not provide quantity of heme iron, the heme iron in animal foods was calculated manually using previously published food composition tables, with information on the average percentage of heme iron in sources of meat products [30]. Subjects were also asked to bring any vitamins/supplements they were currently taking with them to each of the study visits. The ingredient information, including daily dosages of iron, calcium, vitamin C, zinc, and copper, was recorded by the dietitian.

Assessment of Iron Supplement Compliance and Tolerability

A known amount of the assigned iron supplement was provided to each patient, and it was enough to last until the next study visit. Compliance was assessed via pill count and a daily supplement log form, in which patients tracked the times of supplement intake as well as any gastrointestinal related side-effects, reasons for missed doses, and whether the iron supplement was taken with or without food.

At each of the four study visits, participants ranked gastrointestinal-related symptoms (nausea, constipation, diarrhea, vomiting, abdominal pain) on a scale of 1 to 5 (5 being the worst). If the ranking increased at any follow-up visit compared to baseline, then the subject was considered to have experienced a worsening in that symptom.

Assessment of Menstrual Blood Loss

Menstrual blood loss negatively impacts iron status, so this was assessed using a simple visual technique that has been validated previously [37]. Participants recorded the degree to which sanitary wear was soiled, based on a pictorial chart, and the information was used to determine the total volume of blood excreted per cycle.

Statistical Analysis

Data were analyzed using R software version 3.1.2 [38]. The primary outcomes were changes in iron status biomarkers within groups. Secondary outcomes included tolerability and compliance to the iron supplementation, as well as daily dietary and supplemental intake. Power analysis used data from the aforementioned study by Rhode et al., [21] with the caveat that high dose vitamin C was also provided in the Rhode study.. We found that predicting an increase of 12 μg/L in serum ferritin required a sample size of 11 per group (α = 0.05, β = 0.8). With guidance from the Data Safety Monitoring Committee, the study was stopped early because a statistically significant benefit was achieved in the ferrous sulfate group, and no effect was seen in any of the doses of HIP. All three doses of HIP (10.5, 21, and 31.5 mg) were pooled together as one HIP treatment group for analyses of iron status biomarkers (for hemoglobin, sTfR, ferritin, sTfR: ferritin ratios) and plasma hepcidin. This was done after ensuring that for these biomarkers, the residual plots were similar for the reduced (all HIP doses combined) and full (each dose of HIP as separate) models and that the two models were not significantly different. TIBC was excluded from the regression analysis because the three dosages of HIP could be not be pooled together due to differences in the residual plots of the reduced and full models. Two participants in the HIP group, and one participant in the $FeSO_4$ group did not complete the study (see the flow diagram in Fig. 1). We used intention to treat analysis, and missing values were replaced by the last observed value of that variable.

Means and standard deviations were reported for baseline characteristics. These were compared between ferrous sulfate and HIP groups using independent *t* tests. Daily intake of nutrients from food and supplements were compared between the two groups at both baseline and post intervention using the exact Wilcoxon rank-sum test. Within group comparisons of nutrient intakes was done using the exact Wilcoxon signed-rank test. The concentrations of CRP, serum ferritin, sTfR, sTfR: ferritin ratio and plasma hepcidin were log transformed using the natural log prior to statistical analysis to approximate normality, and the results were back-transformed into the original scale. A linear mixed model, controlling for subject level effect, was used to determine the effect of iron supplementation on iron status after 8 weeks. Using the raw rating score of constipation, diarrhea, nausea, vomiting, and abdominal pain reported at each visit, the effects of the two treatments on adverse gastrointestinal side effects were determined using a mixed effects model for ordinal outcome data. Statistical significance was set at $P \leq 0.05$. In the case of the linear mixed effects model (for iron status biomarkers), the least square means were compared within groups and Bonferroni adjustment for two within group comparisons was applied. Thus, differences in least square means were considered significant where $P \leq 0.025$.

Results

Baseline Characteristics of the Study Population

Participant flow is illustrated in Fig. 1. Recruitment began in Fall 2014, and the study ended in Spring 2016. Thirty-one

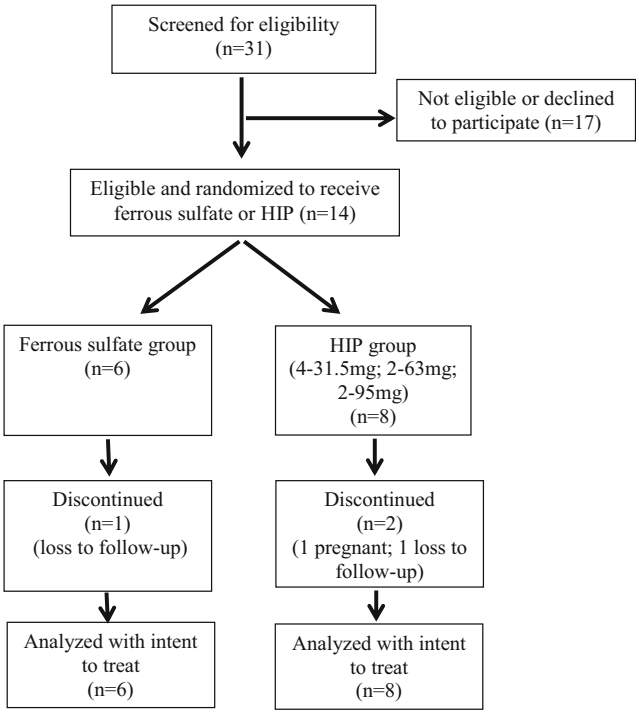

Fig. 1 Flow diagram outlining participants screened, eligible to enroll, and randomized, to receive ferrous sulfate or HIP supplementation during an 8-week, single blind, controlled trial. Patients who dropped out are indicated, but intention-to-treat analysis included all participants

individuals were screened and 14 participants were enrolled in the 8-week study. All subjects were female, and their average age at baseline was 41.5 ± 6.9 years. The average length of time since undergoing RYGB was 6.9 ± 3.1 years. The study population was characterized to be in the mild range of obesity, with a mean BMI of 34.4 ± 5.9 kg/m^2. All participants were classified as iron deficient, meeting at least two of the four diagnostic criteria. Forty-two percent of the entire population suffered from anemia. Six patients were enrolled in the ferrous sulfate treatment group, and eight in the HIP group. Demographic and iron status characteristics (unadjusted values) of patients by treatment group are presented in Table 1. No differences were observed between groups in age, BMI, years since surgery, CRP, plasma hepcidin, or any of the iron status measures at baseline ($P \geq 0.05$). All of the women in the ferrous sulfate group were premenopausal; whereas, 42% of the women in the HIP group were perimenopausal, ($P = 0.05$).

Nutrient Intake of the Study Population

Daily intake of nutrients from food and supplements at baseline and at the final visit, by treatment group, are presented in Table 2. There were no differences between groups in intake of nutrients from food and supplements ($P > 0.05$) at the baseline and final visits, except for the nutrients that patients were instructed to take in their respective treatment group. Thus, compared to baseline, at the final visit, participants in the HIP group increased their intakes of supplemental heme iron ($P \leq 0.016$), and those in the ferrous sulfate group increased their intakes of supplemental non-heme iron ($P = 0.03$). No differences were seen between groups in supplemental calcium and supplemental vitamin C intake, which is expected as all patients were instructed to take the same daily, iron-free, multivitamin /mineral supplement after screening and throughout the duration of the study. Due to multivitamin/mineral supplementation that was implemented throughout the study, deficiencies in other nutrients, besides iron, did not occur.

Influence of Oral Iron Supplements on Iron Status

Blood concentrations of ferritin, sTfR, hemoglobin, and TIBC were assessed at baseline and at 2, 4, and 8 weeks in RYGB patients receiving either ferrous sulfate or HIP supplementation. Table 3 compares the baseline and 8-week values that have been adjusted for person-level random effects. As seen in Table 3, all iron status indicators improved after 8 weeks of ferrous sulfate supplementation ($P < 0.0001$). This was accompanied by a significant increase in plasma hepcidin concentration ($P = 0.006$). Improvements were not observed in any of the iron status indicators following HIP supplementation, neither was there a significant change in plasma hepcidin concentration in this group ($P > 0.025$). For both groups, there was no significant change in CRP concentration after intervention. In accordance to the definition of iron deficiency as having two or more abnormal values, resolution of deficiency occurred in none of the participants receiving HIP, and it occurred in 67% (4 of 6) participants receiving ferrous sulfate supplementation ($P = 0.0094$). After supplementation with $FeSO_4$, anemia was resolved in 75% of the affected participants.

Iron Supplement Compliance and Tolerability

Compliance to taking the iron supplementation was high in both the ferrous sulfate and HIP treatment groups, at 95 and 94%, respectively. The proportion of patients who received $FeSO_4$ and reported worsening of constipation, diarrhea, vomiting, and abdominal pain were 17, 33, 17, and 17%, respectively. In patients receiving HIP, 57, 14, and 14%, reported worsening of constipation, nausea, and abdominal pain, but no changes in vomiting or diarrhea were reported. Although some individuals reported worsening of symptoms following supplementation, differences within or between groups were not significant.

Table 1 Characteristics of patients at baseline in an 8-wk intervention of heme iron (HIP group) or non-heme iron (ferrous sulfate group) supplementation to treat iron deficiency after Roux-en-Y gastric bypass surgery

Characteristic	HIP group (n = 8)	Ferrous sulfate group (n = 6)	P value[d]
Age, years[a]	40.3 ± 8.4	43.2 ± 3.9	0.571
BMI, kg/m[b]	34.0 ± 5.2	34.9 ± 7.1	0.802
Time since surgery, years[a]	6.4 ± 2.0	7.7 ± 4.3	0.475
Iron status measures			
Hemoglobin, g/dL[a]	12.1 ± 0.9	10.8 ± 1.9	0.195
sTfR, mg/L[b]	2345 (1895, 2903)	2653 (1317, 5346)	0.577
TIBC, μg/dL[a]	436 ± 55	415 ± 27	0.329
Ferritin, μg/L[b]	6.6 (4.5, 9.7)[c]	4.9 (2.9, 8.2)	0.155
Plasma hepcidin (ng/mL)[b]	1.65 (1.18, 2.30)	1.63 (1.26, 2.09)	0.937
CRP, mg/L[b]	1.0 (0.5, 1.9)	1.5 (0.7, 3.3)	0.325

BMI body mass index, *sTfR* soluble transferrin receptor, *TIBC* total iron binding capacity, *CRP* C-reactive protein

[a] Values are means ± SDs

[b] Values are geometric means (range of ±1 SD)

[c] Two subjects excluded as outliers

[d] Values refer to comparison between HIP and ferrous sulfate groups

Total Blood Loss per Menstrual Cycle

The volume of blood loss was 58 ± 27 mL per cycle for the ferrous sulfate group, and 32 ± 36 mL for the HIP group, and there was no difference between groups (P = 0.20). Since more women in the HIP group were perimenopausal, and since blood loss was equivalent between groups, this demonstrates that differences in blood loss did not contribute to the

Table 2 Daily intakes of nutrients from food and supplements in patients who took heme iron (HIP group) or non-heme iron (ferrous sulfate group) supplements for 8 weeks

	HIP group (n = 8)		Ferrous sulfate group (n = 6)		P[a]	
	Baseline	Final	Baseline	Final	Baseline	Final
Diet						
Calories (kcal)	1491 (1052, 1988)	1410 (1227, 1994)	1330 (1092, 1738)	1357 (1008, 1920)	0.6623	0.9307
Fat (g)	70 (58, 91)	68 (54, 97)	69 (55, 69)	56 (50, 61)	0.6623	0.6623
Protein (g)	57 (52, 62)	70 (61, 77)	54 (45, 63)	58 (41, 58)	> 0.99	0.5368
Calcium (mg)	817 (550, 1066)	750 (689, 1022)	600 (535, 781)	406 (378, 535)	0.5368	0.1255
Vitamin C (mg)	39 (20, 46)	43 (17, 63)	25 (8, 36)	39 (4, 47)	0.7922	0.5368
Phytic acid	285 (193, 446)	333 (159, 416)	303 (233, 472)	320 (303, 386)	> 0.99	0.7922
Heme iron (mg)	1.4 (0.8, 2.0)	1.9 (1.4, 2.5)	1.1 (1.1, 1.7)	1.3 (1.0, 2.4)	0.7922	0.6623
Non-heme iron (mg)	6.9 (5.2, 10.9)	7.6 (5.3, 11.7)	6.6 (4.8, 7.7)	6.5 (4.2, 8.3)	0.7922	0.5368
Total iron (mg)	9.6 (7.2, 11.9)	9.1 (6.9, 14.5)	7.8 (6.5, 9.6)	7.8 (6.6, 8.9)	0.329	0.5368
Supplement						
Non-heme iron (mg)	0 (0, 0)	0 (0, 0)	0 (0, 0)	193 (186, 193)*	0.4545	0.0022
Heme iron (mg)	0 (0, 0)	30.3 (28.4, 76.5)*	0 (0, 0)	0 (0, 0)	> 0.99	0.0043
Calcium (mg)	200 (200, 673)	200 (200, 200)	200 (171, 200)	143 (143, 200)	0.3182	0.2532
Vitamin C (mg)	75 (75, 75)	75 (75, 75)	75 (64, 75)	54 (54, 75)	0.1212	0.0606

Values are medians (1st and 3rd quartiles)

HIP heme iron polypeptide

*Within group difference from baseline, $P \leq 0.05$

[a] Values refer to comparison between HIP and ferrous sulfate groups

Table 3 Least square means: Iron status changes from baseline to final, after controlling for person-level random effects, in an 8-week intervention comparing ferrous sulfate and HIP supplementation

Group	Biomarker	Baseline	Final	*P* value
$FeSO_4$ ($n = 6$)	Hemoglobin (g/dL)	10.8 (9.8, 11.9)	13.0 (11.9, 14.0)	< 0.0001
	sTfR (μg/L)	2111 (1556, 2864)	1270 (934, 1734)	< 0.0001
	Ferritin (μg/L)	4.9 (3.4, 7.2)	15.5 (10.6, 22.6)	< 0.0001
	sTfR:ferritin ratio	542 (273, 1086)	103 (51, 204)	< 0.0001
	Plasma hepcidin (ng/mL)	1.63 (1.21, 2.18)	2.45 (1.83, 3.28)	0.006
	CRP mg/L	1.53 (0.65, 3.61)	1.66 (0.70, 3.89)	0.81
HIP ($n = 8$)	Hemoglobin (g/dL)	12.1 (11.3, 13.0)	12.2 (11.3, 13.1)	0.597
	sTfR (μg/L)	2345 (1845, 2981)	2528 (1978, 3229)	0.185
	Ferritin (μg/L)	6.6 (4.5, 9.7)	6.1 (4.2, 8.9)	0.575
	sTfR:ferritin ratio	330 (165, 658)	380 (191, 757)	0.428
	Plasma hepcidin (ng/mL)	1.80 (1.40, 2.30)	1.72 (1.35, 2.21)	0.708
	CRP (mg/L)	0.98 (0.48, 2.02)	1.08 (0.53, 2.23)	0.71

Values are means (95% CI). Difference considered significant if $P \leq 0.025$ (Bonferoni adjustment for two comparisons). Baseline and 8-week values have been adjusted for person-level random effects. All variables except hemoglobin were log transformed before linear mixed models were performed, and the least square means (95% CI) were back-transformed into the original scale. Outliers were excluded variable by variable

CRP C-reactive protein, *$FeSO_4$* ferrous sulfate, *HIP* heme iron polypeptide, *sTfR* soluble transferrin receptor

difference in response to iron supplementation that we observed between the ferrous sulfate and HIP group.

Discussion

In this study, we aimed to compare the efficacy of oral supplementation using non-heme iron, in the form of ferrous sulfate, versus heme iron, in the form of HIP, for improvement of iron status in patients who previously had undergone RYGB. Iron status, tolerability, and compliance to the iron supplements were measured during supplementation. The major findings of this study were that ferrous sulfate supplementation at the dose recommended in the clinical practice guidelines is effective in improving iron status. Heme iron supplementation using the HIP formulation appeared to be ineffective. Lastly, we did not find a worsening of gastrointestinal symptoms following supplementation with either formulation.

Our findings support the clinical practice guideline recommendations for treatment of iron deficiency after bariatric surgery. To some extent, our results are surprising given that a previous study showed that RYGB promotes intestinal malabsorption of both non-heme and heme iron [25]. This may be the case under conditions of standard dosing of iron (36 mg iron was used in the previous study [25]), whereas at high doses (195 mg, used in the current study), more iron is absorbed. To our knowledge, no randomized controlled trial has compared oral iron formulations for treatment of iron deficiency in the bariatric surgery population. However, it is worth noting that Brolin et al. [20] tested prophylactic oral iron supplementation for prevention of iron deficiency in women after RYGB. In this prospective, double-blinded study, all patients were randomized to receive either oral iron or placebo, 1 month after RYGB. The investigators demonstrated that prophylactic oral iron supplements were effective in preventing iron deficiency from developing after RYGB. Since we focused our study on patients who were already iron deficient, the findings of our current study extend that of Brolin's to patients who have iron deficiency, and demonstrate that oral supplementation using ferrous sulfate is effective for treatment of iron deficiency after RYGB. If the intervention was longer, we predict that iron deficiency and anemia would resolve in all the participants; however, it is meaningful that, for the majority of participants in the study, resolution of iron deficiency and associated anemia occurred after only 8 weeks of iron supplementation. Interestingly, participants in the study were several years post-surgery, indicating that the study findings could apply to individuals at later timepoints relative to having surgery. In addition, at this post-operative stage, participants were weight stable, which is important since weight fluctuations can affect iron homeostasis.

We assessed diet during the period of iron supplementation but did not control the food intake of participants. We instructed the participants to take a multivitamin and

multimineral (MVM) supplement, throughout the study. Even though diet was not controlled, we found that the dietary intake of both supplemented groups was similar. This outcome may be a consequence of the randomized design of the study, or the fact that both groups consumed an MVM. This ensured that the intakes of nutrients that affect iron absorption, including vitamin C and calcium, were equivalent during the study. In addition, data from our previous study suggests that dietary and supplemental intake of iron and vitamin C are the most important predictors of iron status [30]. This suggests other dietary factors, including calcium, do not play a major role in determining iron status, especially under conditions of high dose iron supplementation.

We also found that HIP was not effective in improving iron status in patients after surgery. The effectiveness of HIP has been evaluated in populations other than bariatric surgery patients. In healthy subjects, the bioavailability of HIP was compared to ferrous fumarate and placebo by measuring the change in serum iron concentrations at 3 and 6 h post ingestion [31]. The findings demonstrated that HIP was more bioavailable than ferrous fumarate when taken with a meal. In a recent review article, Dull et al. [32] reported that the effectiveness of HIP was comparable to that of oral non-heme iron supplementation in chronic kidney disease patients with anemia. These results notwithstanding, our study did not find HIP to be effective in improving iron status after bariatric surgery. While our previous study found that high dietary heme iron is associated with favorable iron status following RYGB [30], the results of the current study do not extend this observation to a positive effect of supplemental heme iron in the form of HIP for treatment of iron deficiency. Since a simple disintegration test showed the HIP formulation was not soluble in water, we speculate that poor solubility may explain the non-effectiveness of supplemental heme in the present study. More research is needed to determine whether heme iron in other formulations could be effective for improving iron status in iron deficient RYGB patients.

A major observation in the Brolin study was that 21% of patients in the oral iron group did not take their iron supplement regularly [20]. In bariatric surgery patients and in the general population, ferrous sulfate supplementation is known to induce adverse gastrointestinal side-effects [29, 39]. These observations suggest that non-compliance is a major issue with oral iron supplementation. For this reason, gastrointestinal adverse events and compliance were strictly monitored in our current study. When comparing from baseline to 8 weeks, we found no significant worsening of nausea, constipation, diarrhea, vomiting, and abdominal pain in subjects taking ferrous sulfate or HIP. The study dietitian offered counseling to participants on ways to alleviate symptoms through diet or nonprescription medication. The lack of adverse events could also be due to the small sample size in our study.

Our study has limitations, the participants, but not the investigators, were blinded to the study intervention, it had a small sample size, and the findings may not be generalizable to post-menopausal women or to men. We purposely recruited younger women because, due to iron loss via the menstrual cycle, this population has a higher risk of developing iron deficiency than post-menopausal women. Also, men account for a relatively small percentage of the total population who undergo bariatric surgery, and also a low percentage of these men develop iron deficiency after the procedure [26]. The study also had a small sample size, which was predicted by power analysis using data from a previous study [21].

Conclusions

Our study is the first to demonstrate that (1) ferrous sulfate supplementation to treat iron deficiency at the dose recommended in the clinical practice guidelines is effective, and (2) heme iron supplementation (in a polypeptide form) is not effective for treating iron deficiency in RYGB patients. Our findings are promising because they have the potential to guide future clinical practice in treatment of iron deficiency after bariatric surgery. In addition, these findings should stimulate future studies on oral iron supplementation in regards to improving tolerability, effectiveness, and compliance.

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Author Contributions NGM and RAM designed research; AB, DJS, and JNC assisted with participant recruitment and translation of study findings; RAM and SMA conducted research; SMA and BAC analyzed data; RAM and SMA wrote the paper; ADR and JNC served as the study physicians; NGM had primary responsibility for final content. All authors have read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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