1 Title: **Influence of diet and supplements on iron status following gastric bypass surgery**
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16 protein; sTfR, serum transferrin receptor
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Abstract

Background: Iron-deficiency is common after Roux-en-Y gastric bypass (RYGB) surgery, but there is no consensus on the optimal diet quality and quantity for restoring and preserving iron status.

Objectives: We explored the impact of dietary and supplemental sources of iron and absorptive factors on iron status.

Setting: Academic, United States

Methods: In a cross-sectional cohort of individuals who have undergone RYGB, dietary intake from food and supplements was measured by three-day food records. Blood biomarkers of iron status, including concentrations of ferritin, total iron binding capacity (TIBC), serum transferrin receptor (sTfR) and sTfR:ferritin, were assessed by a reference laboratory; iron deficiency was defined as having at least two abnormal measures. Associations between iron status biomarkers and dietary predictors were determined using regression analysis.

Results: Participants (N=36) were female (97%), and were age (mean) 45, (95% confidence interval, 41, 48) years, and body mass index 32 (30, 35) kg/m². Forty-two percent of participants were iron deficient. Dietary intake of heme iron, found in meats, was favorably associated with three iron status biomarkers (ferritin, $\beta=0.366$; sTfR:ferritin, $\beta=-0.459$; and TIBC, $\beta=-18.26$, all $P<0.05$), independent of obesity-induced inflammation. Intake of vitamin C from food contributed to iron status (ferritin, $\beta=0.010$, and sTfR:ferritin, $\beta=-0.011$, $P < 0.05$). Use of supplementary non-heme iron, at doses recommended for prophylaxis (45 mg/day), was positively associated with serum ferritin ($\beta=0.964$, $P=0.029$).

39 **Conclusions:** For patients who have undergone RYGB, consuming high, but realistic amounts
40 of heme iron in meat, vitamin C from food, and adherence to recommended iron supplements
41 can prevent iron deficiency.

42 **Keywords:** nutritional complications, iron deficiency, dietary intake, iron supplementation

Introduction

Roux-en-Y gastric bypass (RYGB) bariatric surgery is a proven method for inducing weight loss in individuals who are severely obese, and it can alleviate the burden of type 2 diabetes, cardiovascular disease, cancer, and other obesity-related diseases (1). The benefits of the surgery are mitigated, however, by the high frequency of surgically-induced nutritional complications, which affect a majority of individuals (2). Prophylactic supplementation using multivitamins/ mineral (MVM) formulations is recommended for management of nutritional health (3) and can be effective for vitamin B₁₂ and other B vitamins (4). However, malnutrition in iron, which is an essential trace mineral, is common after RYGB, occurring in 20-50% of patients (2). Also, deficiency in iron is difficult to treat, as many patients do not respond to oral iron supplementation (5, 6). As a consequence of poor nutritional management, patients who have had RYGB often experience anemia, pica, and fatigue (7, 8, 9).

The mechanisms that have been proposed for the worsening of iron status following RYGB are inadequate dietary intake and reduced absorption in the intestine. Intestinal malabsorption of iron following RYGB is documented (10, 11) and may be due to reduced gastric acid secretions, lack of iron transporters due to bypass of the proximal small intestine, and chronic inflammation which is induced by obesity (2). Since iron is poorly absorbed following surgery, the impact of iron obtained from the diet is uncertain. Following RYGB, dietary intake of iron is lower than recommended amounts (12, 13, 14). The bypass procedure promotes early satiety so that food intake is decreased (12). In addition, some patients develop intolerances to red meat, a source of heme-iron which is more bioavailable than non-heme iron (15, 16). Non-heme iron, the inorganic form, is found in plant and animal foods, and is the

standard-of-care formulation used for iron supplementation after surgery. Because intake of iron from dietary sources may be difficult to achieve after surgery, current recommendations suggest that patients supplement their diet with oral iron formulations or MVM supplements that contain iron (3). However, data suggest that oral iron supplementation is not effective for prevention of iron deficiency in gastric bypass patients (6, 17, 18). This may be because the iron content in standard formulations is inadequate or poorly bioavailable (10, 19). It may also be because patients are not well informed about the need to continue taking iron supplements for the rest of their lives to maintain adequate iron status. In addition, patients may not be compliant with the regimen because the supplements are costly or not well-tolerated, since they are associated with nausea and constipation (17, 20, 21). Taken together, the influence of iron ingested from supplements and food sources on iron status in patients who have undergone RYGB is not well understood. Thus, the objective of this study was to evaluate the role of dietary and supplemental intake of iron, as well as factors that inhibit or enhance iron absorption, with regard to iron status in RYGB patients. Using a cross-sectional study design of patients who had already undergone RYGB, we measured dietary intake of heme and non-heme iron, as well as absorptive factors from food and supplements. This information can be used to assess the importance of diet quality - with regard to iron - for the prevention of iron deficiency following RYGB.

Methods

Participants

This was an observational, cross-sectional study in which measurements were prospectively obtained. Study participants were patients who had undergone RYGB, ranging

from 0.6 to 14.3 years previously. Participants were recruited by mailing postcards to 50,000 addresses in the 30-mile radius of Purdue University; these addresses were obtained from a marketing service (Axicom, Little Rock, AR). Thus, participants who enrolled in the study were likely to be representative of a community sample of RYGB patients, unlike other studies using patients who are recruited from a single bariatric center. Patients were eligible if they were between 18 to 65 years of age and had undergone RYGB at least 6 months previously. Patients were ineligible if they were pregnant, had a history of a bariatric surgery revision procedure, had received intravenous iron less than one month previously (22), or had been recently hospitalized due to acute illness. Patients who were taking proton pump inhibitors were eligible for the study, and use of all medications was documented. The study had approval from the IRB Boards of Purdue University (#1111011594) and Indiana University (#1112007617) and is registered with ClinicalTrials.gov, NCT011775579.

Clinical Measurements

Participants attended a research visit with the study team in the morning after undergoing an 8-hour overnight fast. Participants removed shoes for height and weight measures. Information regarding demographics and relevant medical and surgical history was obtained during an interview. A blood draw was obtained using venipuncture, and blood was collected for analysis of Fe, Zn, and Cu, as well as associated nutrients (vitamins B₆, B₁₂, and folate). The following laboratory assays were undertaken by Mid America Clinical Laboratories (MACL, Indianapolis, IN, USA), a reference laboratory, using methods that are available (23); 1) total iron binding capacity (TIBC) was measured using the tripyridyltriazine/ferine method; 2) ferritin, vitamin B₁₂, and folate were measured using chemiluminescent microparticle immunoassays; 3) Cu and Zn were assessed in blood collected in tubes that were metal free (Royal Blue, Becton,

Dickinson and Company, Franklin Lakes, NJ, USA) and measured using inductively-coupled plasma mass spectrometry; 4) vitamin B₆ from serum was measured using liquid chromatography coupled to tandem mass spectrometry; 5) hemoglobin was assessed using a hematology analyzer (ADVIA® 2120i, Seimens, Henkestrasse, Germany); and 6) high sensitivity C-reactive protein (CRP) was measured using the turbidimetric latex method. Serum transferrin receptor (sTfR) and plasma hepcidin concentration were measured in the laboratory using an enzyme-linked immunosorbent assay kits from R&D Systems (Minnesota, MN, USA) and DRG International Inc. (Springfield, NJ, USA). Intra-assay coefficients of variation for the two assays were 2.4% and 9.1% respectively. For each assay, the control values were within the provided range.

Unless stated otherwise, normal reference values were determined by MACL. Because a sole, reliable biomarker for the diagnosis of iron deficiency does not exist, the best assessment of iron status combines several indicators (24). Subjects were found to be deficient in iron if they met at least two of the following conditions: TIBC > 370 µg/dL, serum ferritin < 20 µg/L (24, 25), serum transferrin receptor (sTfR) > 28.1 nmol/L, sTfR:ferritin ratio > 500 (25). Subjects were anemic if hemoglobin < 12 g/dL in menstruating women, and hemoglobin < 13 g/dL in men and post-menopausal women (26). Deficiencies in other nutrients were determined using the following cut-offs: Cu < 70 µg/dL; Zn < 60 µg/dL; vitamin B₆ < 2.1 ng/mL; folate ≤ 5.4 ng/mL; and vitamin B₁₂ < 211 pg/mL. We assessed the status of these nutrients because they share signs and symptoms with deficiency in iron, such as anemia.

Assessment of Dietary Intake

Immediately prior to the study visit, participants recorded dietary intake using three-day food and supplement records, which consisted of two weekdays and one weekend day. During

the study visit, the study dietitian reviewed the records with the participant for accuracy and completeness. Participants were asked to bring any nutritional supplements that they were currently taking with them. The ingredient information, including daily dosages of vitamin and minerals, was recorded by the study dietitian. Food records were analyzed using Nutrition Data System for Research software, version 2013 (University of Minnesota, Minneapolis, MN, USA), and average daily intake was calculated. We were specifically interested in dietary composition of iron, as well as vitamin C, phytate and calcium, since the latter nutrients enhance or inhibit iron absorption in the intestine (27). However the analysis did not provide any detail of the composition of heme and non-heme iron in the diet. Therefore, the composition of heme and non-heme iron in animal foods was determined using previously published food composition tables, with information regarding the percentage of heme iron in different sources of meat (28). Using these composition tables, for example, the mean percentage of heme-iron used for all types of chicken products (i.e. chicken breast, chicken leg, chicken thigh, etc.), was 28% of the total iron in the food. Similarly, the mean heme-iron percentage of all pork products was 61%; for all beef products, it was 78%; for all turkey products, it was 35%, and for all fish products it was 35% of the total iron.

Statistical Analysis

Data were analyzed using R software version 3.1.2 (29). Wilcoxon rank sum tests were conducted to compare nutrient intake among participants who were deficient in iron versus those who were sufficient. All other variables were compared between the two groups using independent t-test. BMI and the concentrations of serum ferritin, serum sTfR, sTfR: ferritin ratio, serum CRP, plasma hepcidin and serum iron were log transformed using the natural log prior to statistical analysis to approximate normality and the geometric means were reported for these

variables. Medians were reported for nutrients intake and the means were reported for all other variables. Interquartile ranges (IQRs) were reported for nutrient intakes as a measure of variability, while the 95% confidence intervals (CI)s were reported for all other variables. Fisher's exact test was used to determine proportional differences. Partial correlations were used to probe the associations among BMI, plasma hepcidin, serum ferritin and serum CRP among the entire population. Also for the total group, associations among iron status biomarkers and nutrient intake from food and supplements were assessed using multiple linear regression. The iron status biomarkers included as outcome variables for the different regression models were serum concentrations of ferritin, sTfR, TIBC, and sTfR:ferritin. Nutrient intakes of heme and non-heme iron, vitamin C, protein and phytate from food, and supplemental intakes of calcium and vitamin C were included as independent variables in each regression model. Consumption of at least 45 mg/day of non-heme iron supplement was defined as use of supplemental iron and this was included in the regression model as a dichotomous variable. We included this variable since guidelines for prophylactic iron supplementation suggest 45 to 60 mg iron/day (3). To control for obesity-induced inflammation, CRP was included in the models. Stepwise backward elimination using the Akaike Information Criterion (AIC) method, was applied for variable selection. Results of the linear regression were presented only for biomarkers that showed significant associations with dietary factors. Statistical significance was set at $P \leq 0.05$.

Results

Characteristics of the Study Population.

Thirty-six participants were eligible and completed all study measures. Within the participant pool, over six different bariatric surgery centers were represented. All participants

self-reported their race/ethnicity as non-Hispanic White. Most subjects were female (97%), and their average age at the time of the study was 45 years (95% CI, 41, 48 years). Seventy-two percent of the population had not undergone menopause. The length of time since participants had undergone RYGB was 6.8 years (5.4, 8.2 years). The population was characterized to be in the low range of obesity, with a BMI of 32 kg/m² (30, 35 kg/m²). Forty-two percent of participants were classified as iron deficient, meeting at least 2 of the 4 diagnostic criteria. Forty-four percent of the entire population suffered from anemia, and twenty-eight percent of the participants were both iron deficient and anemic. The prevalence of deficiency in zinc, copper, vitamins B₆, and folate were 17%, 3%, 9% and 3% respectively. There was no case of vitamin B₁₂ deficiency among study participants.

Demographic and clinical characteristics of subjects with regard to their iron nutritional status are presented in **Table 1**. There were no differences in demographic characteristics between iron deficient and iron sufficient participants ($P > 0.05$). However, per the diagnostic criteria, iron deficient patients had abnormal concentrations of biomarkers for iron status including serum concentrations of ferritin, sTfR, TIBC, and the sTfR/ferritin ($P < 0.01$) (Table 1). Hepcidin concentration was higher in the iron sufficient group ($P < 0.0001$), although CRP concentrations were not different between the two groups ($P = 0.78$).

Nutrient Intake of the Study Population.

Daily intake of nutrients from food and supplements are presented in **Table 2**. With the exception of intake of protein ($P = 0.03$), supplemental nonheme iron ($P = 0.01$), total iron ($P = 0.005$), and total vitamin C ($P = 0.02$) which were lower in the iron deficient group, intake of nutrients were similar between the two groups ($P > 0.05$). Thirty-six percent of the total population used supplements that contained at least 45 mg/d of non-heme iron to a maximum of

325 mg/d. Iron deficiency was present in 57% of non-users of iron supplements whereas only 15% of iron supplement users had iron deficiency ($P = 0.03$). Also, between users and non-users of iron supplements, users had higher serum ferritin, and lower TIBC and sTfR:ferritin than non-users ($P \leq 0.05$), signifying better iron status among supplement users (data not shown).

Associations between Nutrient Intake and Iron Status.

Relationships between nutrient intake and iron status were explored using multiple linear regression analysis and findings are reported in **Table 3**. Dietary intake of heme iron from foods was positively associated with serum concentrations of ferritin ($P = 0.005$), and negatively associated with sTfR:ferritin ratio and TIBC ($P < 0.01$). Intake of vitamin C from foods was also positively associated with serum ferritin ($P = 0.01$), and negatively associated with sTfR:ferritin ($P = 0.018$). Supplemental non-heme iron use was associated with higher serum ferritin concentration ($P = 0.029$). No associations were observed between intake of other nutrients from food (*e.g.* non-heme iron, phytate) or supplements (*e.g.* calcium) and any of the biomarkers of iron status.

Effects of Obesity and Inflammation

We wished to determine if the observed relationships between nutrient intake and iron status were modulated by chronic inflammation due to obesity. This may not be an issue for the population in the current study since they are characterized as mildly obese and non-inflamed. Since iron status can be modulated by obesity-induced inflammation via the activity of hepcidin, we explored relationships among BMI, and systemic concentrations of ferritin, hepcidin, and CRP (**Table 4**). Correlation analyses showed a positive relationship between plasma hepcidin and ferritin, even after controlling for BMI and CRP ($r = 0.94$; $P < 0.0001$). In fact, obesity or

inflammation did not impact hepcidin or ferritin since correlations between these variables were not significant. Thus, we can conclude that the relationship between nutrient intake and iron status among subjects in this study is independent of obesity-induced inflammation. This assertion is further supported by the fact that CRP was not significantly associated with any of the iron status biomarkers in the regression models and was thus excluded in the process of variable selection. A positive correlation between BMI and CRP, independent of hepcidin and ferritin concentrations, was also observed ($r = 0.44$, $P = 0.009$), which demonstrates the well-established relationship between obesity and chronic inflammation.

Discussion

In this study, we aimed to identify factors in the diet that influence iron status in patients who have undergone RYGB. We explored cross-sectional relationships between dietary intake of iron and its absorptive factors, from food and supplement sources, and several biomarkers of iron status. The major findings of this study were that dietary intake of heme iron, which is high in meat products, was the dietary factor that was most related to iron status following RYGB. Vitamin C, obtained from foods, and supplemental intake of non-heme iron, at the daily dose recommended, were also associated with improved iron status. The relationships between dietary factors and iron status were not influenced by obesity-induced inflammation.

Our finding that heme iron intake is a strong determinant of iron status is novel. To our knowledge, no studies have explored the role of ingestion of iron from foods on iron status in patients who have undergone RYGB; instead a few studies have focused on the impact of iron obtained from dietary supplements (6, 12, 18, 30). The strength of the relationship between dietary heme iron and iron status was evident by the fact that intake of heme iron was consistently related to several biomarkers of iron status (ferritin, TIBC and sTfR:ferritin ratio). In addition, this relationship remained strong despite correcting for intake of selected nutrients which are known to influence iron status, such as vitamin C (which enhances iron absorption). We found that intake of non-heme iron from supplements was strongly related to serum ferritin concentrations. In normal healthy individuals, evidence is well-established that intestinal absorption of heme iron is superior to non-heme iron (27). This is because the bioavailability of non-heme iron is affected by enhancers such as vitamin C and inhibitors such as phytates. It is therefore not surprising that dietary heme iron was more prominently associated with biomarkers of iron status than non-heme iron and our findings in individuals who have undergone RYGB,

mirror what has long been observed in the general population. Non-heme iron is present in MVM formulations at various doses, but we only compared the effects of iron supplementation between those who were taking the dose of iron which is prescribed for patients after RYGB (45 mg/day) and those taking less than this dose. Given the relatively small sample size in this study and the fact that nearly half (42%) of subjects did not take iron supplement at all, we believe that the current study is not sufficiently powered to test a hypothesis regarding the effectiveness of various doses of iron supplementation. Although we found a positive impact of non-heme iron supplementation on iron status, findings in the literature have been mixed with regard to this issue. Some studies have found that prophylactic iron supplementation was effective for prevention of deficiency (31) but other studies found no benefit (6, 17, 18).

The other nutrient that emerged to be a strong contributor to iron status was vitamin C. Interestingly, intake of vitamin C from food sources was associated with improved serum ferritin and sTfR:ferritin, whereas vitamin C from supplements was not associated with any iron status biomarker. Although participants ingested more vitamin C from supplements than they did from foods, only intake from foods had a significant impact. The reason that vitamin C within food would better facilitate iron absorption compared to supplemental sources is not clear. We speculate that vitamin C in food will better enhance the absorption of non-heme iron since it is likely to be a component of the iron-containing meal, whereas the effect of supplemental vitamin C will depend on whether it is consumed with the meal. Additionally, formulations of vitamin C, when found within MVM or in isolation, would be susceptible to issues regarding solubility and bioavailability in the small stomach pouch that is created with RYGB.

It has been shown previously that intestinal absorption of both heme and non-heme iron is reduced following RYGB (to 26% and 42% of pre-surgery values, respectively) (10). Since

iron absorption is shown to be impaired so dramatically, our finding that dietary intake of heme iron and supplemental non-heme iron predicted iron status was surprising. Unlike the studies by Ruz and colleagues, we assessed the exposure of iron through diet versus that from a single meal, and we believe that our diet-based assessment represents the free-living situation. It is encouraging that dietary intake of heme iron and vitamin C, and supplemental intake of non-heme iron were associated with iron status after RYGB, even in the context of reduced absorption due to bypass of the proximal intestine or obesity-induced inflammation. This finding is important for those who have had RYGB because dietary intake is modifiable although reduced absorption may not be, since the surgical procedure may induce physiological changes in gastric acid secretion and access to iron transporters that are long-lasting (10). Thus, our findings highlight the unique roles of dietary heme iron and vitamin C, and supplemental intake of non-heme iron for maintaining iron nutrition after surgery, despite the negative impact of RYGB on intestinal iron absorption. Since heme iron is uniquely found in animal foods, particularly meats, our findings suggest that high dietary intake of meat could protect against iron deficiency. Unfortunately, many patients may not be able to increase their intake of heme iron because of intolerance to red meat that has been demonstrated to develop post-surgery (15, 16). Other meat sources of heme iron, however, will be helpful in addressing iron deficiency after RYGB. Based on the effect sizes determined, among iron deficient subjects, increasing heme iron and vitamin C intakes from food by 0.5 mg/d and 20 mg/d respectively, in addition to consuming an iron supplement of at least 45 mg/day, will improve serum ferritin concentration above the threshold of 20 µg/L. If we incorporate these suggestions into the diet of the iron deficient group, individuals would need to consume a total of 1.7 mg/d of heme iron and 37 mg/d of vitamin C, both of which are realistic for RYGB patients to achieve. A total of 1.7 mg/d

heme iron can be easily split up into 3 meals (each containing 0.57 mg heme iron and such meals can be either 7 oz chicken, 4.5 oz turkey, 1 oz beef, or 5 oz pork). The amount of vitamin C required, 37 mg, can be obtained simply by consuming ½ cup cooked broccoli, 1 medium orange, or ½ cup fresh sliced strawberries. A caveat of these assumptions are that other factors including blood loss through menstruation, status of other nutrients and gastrointestinal complications may also influence iron status. Future interventional studies should test the effectiveness, safety, and tolerability of increasing intake of foods high in heme iron for the prevention of iron deficiency in patients who have undergone RYGB.

Our study has limitations, most of which relate to the fact that it was observational, and thus our finding of associations cannot be interpreted as cause and effect. In addition, although we aimed to recruit both males and females, most of the participants were female and all were of white racial background. Thus, our findings may not be generalizable to men or non-white populations. Male gender is not a risk factor for iron-deficiency post RYGB (18), since men do not lose iron through menses as women do. Another limitation was that one-third of the participants in the study used proton pump inhibitors and H2 receptor antagonists, but the effect of these medications on iron status was not determined. Because proton pump inhibitors have been shown to reduce the secretion of gastric acid (32), use of these medications may confound the results of the study. The small sample size of 36 may be considered a limitation as well. This may account for the lack of significant differences between iron deficient and iron sufficient subjects in some nutrient intakes. Also the small sample size limited the ability to assess other confounding variables such as length of time following surgery. A future study with larger sample size and with a longitudinal design is therefore recommended. Lastly, we acknowledge limitations associated with the collection of dietary intake data. Dietary intake data in this study

was obtained using three day food records which could influence subjects' food intake on days of the record. However, this method is well-accepted as a gold-standard method for obtaining dietary intake data (33). Moreover, dietary records were obtained for two weekdays and one weekend to improve representativeness of subjects' habitual intake, and they were carefully reviewed for completeness and accuracy by the study dietitian.

Our study is the first to demonstrate that high intakes of heme iron and vitamin C as well as compliance to the minimum dose recommended for prophylactic iron supplementation are associated with improved levels of selected iron status biomarkers for patients undergoing RYGB, regardless of low grade, obesity-induced inflammation. Our finding is promising because it suggests that within intakes that are realistic for RYGB patients, modulation of diet to include foods high in heme iron and vitamin C and adherence to recommendations of iron supplementation can overcome the detrimental impact on iron absorption induced by gastric bypass surgery. Taken together, these findings should stimulate studies of interventions that test the effectiveness of high quality sources of foods and supplements for the prevention of iron deficiency following gastric bypass surgery.

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Conflict of Interest

The authors do not have any conflicts of interest to declare.

Contribution of Co-Authors

RAM conducted the clinical study procedures, performed the dietary analysis, and contributed to the writing of the manuscript; SMA assisted with dietary analysis, conducted the statistical analysis and contributed to the writing of the manuscript; BNW conducted the clinical study procedures and contributed to the writing of the manuscript; APR served as the study physician, and contributed to the interpretation of the data; SGM assisted with patient recruitment, provided oversight at the Indiana University Clinical Research Center, and contributed to the writing of the manuscript; NGM provided oversight of all aspects of the study, including conceptual design, interpretation of findings, and writing of the manuscript. All authors contributed to the revision of the manuscript and approved of the final draft.

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Table 1: Participant characteristics and iron status biomarkers of RYGB surgery patients post-surgery (n=36)¹

	Iron sufficient (n=21)	Iron deficient (n=15)	P-value
Age, year	44 (39, 48)	46 (41,51)	0.41
Time since surgery, year	6.2 (4.1, 8.3)	7.6 (5.7, 9.6)	0.29
Menopause ² , n (%)	6 (29)	4 (27)	0.99
BMI ² , kg/m ² , ³	34 (30, 37)	30 (28, 34)	0.14
Hemoglobin, g/dL	13.0 (12.6, 13.3)	11.5 (10.7, 12.4)	0.004
Serum ferritin ³ , µg/L	46 (27, 800)	6 (5, 7)	<0.0001
Serum sTfR ³ , nmol/L	14.7 (12.6, 17.2)	26.8 (22.1, 32.6)	<0.0001
sTfR/ferritin ratio ^{2,4}	27 (15, 50)	406 (284, 578)	<0.0001
TIBC, µg/dL	311 (295, 327)	423 (405, 442)	<0.0001
Serum iron ³ , µg/dL	77 (64, 94)	37 (26, 51)	0.004
Plasma hepcidin ³ , ng/mL	7.8 (5.5, 11.2)	2.4 (2.1, 2.8)	<0.0001
Serum CRP ³ , mg/L	1.0 (0.6, 1.7)	1.1 (0.7, 1.7)	0.78

¹ Values are means (95% CIs). CRP, C-reactive protein; RYGB, roux-en-Y gastric bypass; sTfR, soluble transferrin receptor.

²P-value based on Fisher's exact test

³Values are geometric means (95% CIs).

⁴sTfR values were converted to µg/L using conversion factor 1nmol/L = 84.7 µg/L before the sTfR/ferritin ratio was calculated

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Table 2: Nutrient intakes among RYGB patients post-surgery (n=36)¹

	Iron sufficient	Iron deficient	P-value
Energy, kcal/d	1702 (1395, 2250)	1224 (918, 1733)	0.08
Protein, g/d	73 (59, 94)	50 (44, 69)	0.03
Fat, g/d	76 (57, 92)	44 (36, 86)	0.11
Heme iron, mg/d	1.2 (0.7, 1.9)	1.2 (0.8, 1.6)	0.87
Non-heme iron, mg/d	10.6 (7.0, 13.7)	7.4 (4.9, 11.2)	0.21
Supplemental nonheme iron, mg/d	50 (0, 65)	0 (0, 18)	0.01
Total iron, mg/d	66 (14, 79)	11 (7, 30)	0.005
Vitamin C, mg/d	48 (34, 89)	17 (10, 63)	0.10
Supplemental vitamin C, mg/d	113 (0, 180)	30 (0, 88)	0.06
Total vitamin C	160 (81, 321)	92 (14, 141)	0.02
Phytate, mg/d	461 (271, 763)	322 (212, 497)	0.29
Supplemental calcium, mg/d	300 (0, 1300)	200 (0, 419)	0.28

¹Intakes are from food sources except where indicated as supplemental. Values are medians
(inter-quartile ranges).

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503 Table 3. Associations between biomarkers of iron status and nutrient intake in RYGBpatients¹

	Serum ferritin			sTfR:ferritin ratio			TIBC		
	μg/L						μg/dL		
	β	SE	P	β	SE	P	β	SE	P
Heme iron, mg/d	0.366	0.122	0.005	-0.459	0.155	0.006	-18.26	5.87	0.004
Vitamin C, mg/d	0.010	0.003	0.010	-0.011	0.004	0.018	-0.32	0.17	0.063
Supplemental non-heme iron use, (Yes)	0.964	0.421	0.029	-1.071	0.535	0.054	-34.29	20.78	0.109
Supplemental vitamin C, mg/d							-0.08	0.04	0.057

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505 ¹Stepwise backward elimination was used for variable selection in multiple linear regression

506 models. Serum ferritin and sTfR were log transformed prior to regression analysis. Nutrient

507 intakes of heme and non-heme iron, vitamin C, protein and phytate from food, and supplemental

508 intakes of calcium, vitamin C and non-heme iron were included as independent variables in the

509 regression models. Supplemental non-heme iron intake was included as a dichotomous variable

(≥ 45 mg/d non-heme iron from supplement as “Yes”). Values that are depicted in bold were significant in the regression analysis, $P \leq 0.05$. sTfR, soluble transferrin receptor; TIBC, total iron binding capacity. Based upon effect sizes determined, increasing heme iron and vitamin C intake from foods by 0.5 mg/d and 20 mg/d, respectively, in addition to consuming an iron supplement of at least 45 mg/day, will improve serum ferritin concentration above the threshold of 20 $\mu\text{g/L}$.

522 Table 4. Partial correlations among markers of iron status, inflammation and adiposity in RYGB

523 patients (n=36)¹

	CRP	Serum ferritin	BMI
	mg/L	µg/L	kg/m ²
Plasma	0.18 (0.30)	0.94	0.079
hepcidin,		(<0.0001)	(0.66)
ng/mL			
Serum CRP,	—	-0.21	0.44
mg/L		(0.23)	(0.009)
Serum	—	—	-0.018
Ferritin ² , µg/L			(0.92)

524 ¹Values are r (P-value). All variables were log-transformed prior to correlation analysis.

525 Correlations were performed on each pair of variables, controlling for the other two variables.

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