Iron nutriture following roux-en-y gastric bypass surgery

Breanne N. Wright

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By Breanne Wright

Entitled
IRON NUTRITURE FOLLOWING ROUX-EN-Y GASTRIC BYPASS SURGERY

For the degree of Master of Science

Is approved by the final examining committee:

Nana Gletsu-Miller

Dorothy Teegarden

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Nana Gletsu-Miller

Approved by Major Professor(s): ________________________________

Connie Weaver 07/11/2014

Head of the Department Graduate Program Date
IRON NUTRITURE FOLLOWING ROUX-EN-Y GASTRIC BYPASS SURGERY

A Thesis

Submitted to the Faculty

of

Purdue University

by

Breanne N. Wright

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

August 2014

Purdue University

West Lafayette, Indiana
I dedicate this thesis to my family, for their endless love and support.
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<td>Liquid Chromatography Coupled to Mass Spectrometry</td>
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<td>MCH</td>
<td>Mean Corpuscular Hemoglobin</td>
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<td>Mean Corpuscular Volume</td>
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<td>NASH</td>
<td>Nonalcoholic Steatohepatitis</td>
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<td>NDSR</td>
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<td>RYGB</td>
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<td>SAT</td>
<td>Subcutaneous Adipose Tissue</td>
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<td>sTfR</td>
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ABSTRACT

Wright, Breanne N. M.S., Purdue University, August 2014. Iron Nutrition following Roux-en-Y Gastric Bypass Surgery. Major Professor: Nana Gletsu-Miller.

Roux-en-Y gastric bypass surgery (RYGB) is effective for weight loss, but is commonly associated with iron deficiency and its clinical manifestation, anemia. Diagnosing iron deficiency is complex because iron status depends on other nutrients; additionally, anemia following surgery is not specifically due to deficiency in iron, as it can be due to deficiencies in other nutrients including zinc, copper, vitamin B₆, folate, and vitamin B₁₂. In patients who have undergone RYGB, our aims were to 1) conduct a comprehensive assessment of nutrients involved in iron homeostasis, 2) determine the contribution of dietary intake to iron deficiency, and 3) describe associations between anemia and nutritional status of iron and other nutrients. Systemic measures of hemoglobin, ferritin, serum transferrin receptor (sTfR), total iron binding capacity (TIBC), copper (Cu), vitamins B₆ and B₁₂, folate, zinc (Zn), and C-reactive protein (CRP) were determined using reference methods. Iron deficiency equaled having ≥ 2 abnormalities in: ferritin, sTfR, sTfR:ferritin, or TIBC. Ferritin, a measure of iron stores, was defined as normal (ferritin ≥ 20 mcg/L) or low (ferritin < 20 mcg/L). Statistics included prevalence, mean ± standard error of the mean (s.e.m.) for normally-distributed data, median ± semi-interquartile range for skewed data
frequency tables, t-tests (independent, by group), correlations, and general linear models (significant if p < 0.05). Subjects (N=70) were 91% female, age 49 ± 1 years, 4 ± 2 years post surgery, and 79% Caucasian. Fifty-six percent of the total population and 96% of the subpopulation with anemia (N=26) presented with deficiencies related to iron nutriture, including deficiencies in iron, zinc, copper, vitamin B₆, folate, and vitamin B₁₂. The most prevalent nutrient deficiencies in the total population and the subpopulation with anemia were iron and zinc; prevalence of iron and zinc deficiency in the total population was 24.3% and 20.0%, respectively, and prevalence of iron and zinc deficiency in the subpopulation with anemia was 46.2% and 23.1%, respectively. Participants in the total population and the subpopulation with anemia were also deficient in copper, vitamin B₆, vitamin B₁₂, and folate (11.5% and 26.9%, respectively). In the total population, iron and zinc deficiency occurred in isolation and also in combination with other nutrient deficiencies; all other nutrient deficiencies occurred in combination. In the subpopulation with anemia, only iron deficiency occurred in isolation. The dietary intake of the study population exceeded the RDAs for all nutrients assessed. In addition, patients with low ferritin concentrations consumed lower total energy (p= 0.009), fat (p= 0.026), protein (p=0.013), and animal protein (p=0.023), compared to patients with normal ferritin concentrations. Dietary intake of heme iron was correlated with years post-RYGB surgery (r=0.67, p<0.05). In conclusion, in a community-based surveillance of RYGB patients we found that, more often than not, RYGB patients presented with micronutrient deficiencies related to iron nutriture; this includes
deficiencies in iron, copper, zinc, vitamin B₆, folate, and vitamin B₁₂. It was more likely for multiple deficiencies to occur simultaneously than for deficiencies to occur alone. In addition, there was a high prevalence of anemia, a clinical manifestation of deficiencies in these nutrients. Implications of these findings are that clinicians who evaluate post-RYGB patients, especially patients who present with known symptoms or manifestations of iron deficiency, should also screen patients for deficiencies in copper, zinc, vitamin B₆, folate, and vitamin B₁₂, as these deficiencies are also prevalent following surgery, may occur simultaneously with iron deficiency, and are also implicated in anemia. Based on our findings that RYGB patients who have more favorable iron stores consume more energy, fat, and protein than patients with low iron stores, increasing protein intake following surgery may improve iron status; although, increasing consumption of fat may have detrimental effects on weight regain in the RYGB population. Findings of this study may be used to enhance prophylactic measures and treatments for iron deficiency following RYGB.
CHAPTER 1. INTRODUCTION

1.1 Obesity and its Health Consequences

Obesity is a significant health problem resulting in many comorbidities including diabetes, cardiovascular disease and cancer.\(^1\) Rates of moderate and severe obesity are continuing to rise in the United States.\(^2\) Although obesity is generally thought of as a condition of overnutrition, overweight and obesity are also known risk factors for deficiencies in various micronutrients, including vitamins A, E, B\(_1\) and B\(_{12}\), folate, selenium, chromium, zinc, and iron.\(^3,4\) In particular, a high prevalence of iron deficiency is documented in the severely obese population (28\%-35\%).\(^5-7\) Bariatric weight loss surgery is growing in popularity as an effective, long-term treatment for moderate and severe obesity, but is also associated with iron deficiency (postoperative prevalence: 15\%-54\%).\(^8-12\). This chapter discusses iron deficiency, factors that contribute to iron homeostasis, the clinical manifestation of anemia, mechanisms for iron deficiency in obesity, and mechanisms for iron deficiency following bariatric surgery.
1.2 Iron Deficiency and Homeostasis

1.2.1 Iron Deficiency

Iron deficiency is the most common and widespread nutritional disorder in the world, and the only nutrient deficiency that is significantly prevalent in industrialized nations.\textsuperscript{13,14} It is associated with increased infant mortality and perinatal risks for mothers and neonates, it adversely affects cognitive performance, behavior, and physical growth of infants and adolescents, as well as immune status, morbidity from infections, physical capacity, and work performance in all age groups.\textsuperscript{13} Therefore, patients who develop iron deficiency have a decreased quality of life. Severe iron deficiency is associated with low blood concentrations of hemoglobin, and is termed iron deficiency anemia (IDA).\textsuperscript{13}

1.2.1.1 Causes

The causes of iron deficiency are multifactorial. Iron deficiency may be caused by blood loss due to disease, infection, hemorrhage in childbirth, menstruation, or trauma. Iron deficiency may also be nutrient-related as a consequence of poor diet quality, such as low intake of bioavailable iron, deficits in specific nutrients, e.g. copper, vitamin B\textsubscript{6} and zinc which are required for iron absorption and heme synthesis, or high concentrations of nutrients that compete with iron for absorption. The cause of iron deficiency may also be systemic,
referring to the physiological state of the host, such as inflammation and/or mechanical alterations due to gastrointestinal surgery.4

The multifactorial etiology of iron deficiency includes true and functional iron deficiency. The former is characterized by reduced iron stores, while the latter describes a compromised supply of iron to tissues and/or mobilization of iron between tissues. Functional iron deficiency can be affected by conditions such as obesity-induced inflammation and thus is highly dependent on the systemic state of the individual.

1.2.2 Iron Homeostasis

Because of iron's central role in oxygen and energy metabolism, iron absorption, utilization and storage are tightly regulated to maintain homeostasis. Unlike other essential elements, iron is highly conserved. Total iron body content is estimated to be 3800 mg in men and 2300 mg in women; relatively small amounts of iron are lost in feces (~0.6 mg/day), urine (<0.1 mg/day) and sweat (<0.3 mg/day).15 Because there are no other excretion routes for iron and iron is mainly lost through blood, fecal iron content can be used to indirectly measure iron absorption. Approximately 20 to 25 mg of iron is recycled daily with the erythrophagocytosis of senescent red blood cells in the spleen, and iron that is released from heme is captured and reutilized to produce new erythrocytes in bone marrow.15 The body also contains a large storage pool for iron (in its storage form- ferritin), mainly in the liver, spleen and bone marrow.
1.2.2.1 Intestinal Iron Absorption

1.2.2.1.1 Heme and Non-Heme Iron

There are two bioavailable forms of iron that are obtained from the diet: heme and non-heme iron. The more bioavailable heme iron is found in meat, fish and poultry; about 40% of the iron is bound into heme while the other 60% is non-heme iron. The form of iron primarily found in grains, vegetables and legumes is non-heme iron. For meat-eating individuals, heme iron makes up about two-thirds of the iron that is absorbed. Heme iron is soluble at the pH of the duodenum where most iron absorption occurs, and is not affected by the dietary constituents that impair non-heme iron absorption such as phytates, polyphenols, oxalates, calcium and other divalent cation minerals. Co-consumption with vitamin C - rich foods, and meat, fish and poultry enhances non-heme iron absorption. There is a high incidence of iron deficiency anemia in countries where meat is less available because of the predominance of non-heme iron-containing foods in the diet.

1.2.2.1.2 Mechanism for Intestinal Iron Absorption

Iron homeostasis is controlled at the level of intestinal absorption. Iron absorption is an important determinant of iron homeostasis and is highly regulated. Iron absorption increases when iron stores are low and decreases when iron stores are high. Uptake of non-heme iron by enterocytes is better
understood than heme iron. The abbreviated mechanism of iron passage across the enterocyte is shown in figure 1.1:\textsuperscript{28}

Figure 1.1 Iron Passage Across the Enterocyte. Ferric iron is reduced by the brush border ferric reductase, Dcytb, before being transported into the cell via Dmt1. Iron is either stored as ferritin or exported into the circulation across the basolateral membrane via Fpn1. The iron oxidase, Hp, converts ferrous iron to ferric iron for efficient export. Iron circulates bound to Tf. Liver-derived Hepcidin reflects systemic iron demands, and removes Fpn1 from the plasma membrane in order to prevent iron export.

First, the membrane-bound ferroxidase duodenal cytochrome b (Dcytb) reduces ferric iron (Fe\textsuperscript{3+}) to ferrous iron (Fe\textsuperscript{2+}). Then, divalent metal transporter-1 (Dmt1) transfers molecular Fe\textsuperscript{2+} from the luminal side into the enterocyte. Iron entering the enterocyte is either incorporated into ferritin, the storage form of iron, or subjected to basolateral export by transmembrane protein ferroportin 1 (Fpn1). Ferrous iron then undergoes oxidation by the membrane-bound copper-containing ferroxidase hephaestin (Hp) before being incorporated into transferrin, the mobile form of iron, and entering circulation.\textsuperscript{29} It is important to note that, in
addition to intestinal absorptive cells, ferroportin is on the surface of hepatocytes, macrophages and placental cells, all of which release iron into the plasma.\textsuperscript{30} Hepcidin is a liver-derived peptide implicated in the control of iron homeostasis. Hepcidin inhibits iron absorption by binding to ferroportin, targeting its degradation, and ultimately blocking iron efflux.\textsuperscript{28,29} Therefore, hepcidin blocks intestinal iron absorption and release in various cell types. Genes associated with hereditary hemochromatosis (a genetic disease causing the body to absorb too much iron), \textit{HFE} and \textit{TFR2}, have been found to regulate hepcidin levels through an iron-sensing mechanism.\textsuperscript{15,31-33}

1.2.2.2 Heme Synthesis

Most body iron is found as heme iron; heme is the essential constituent for oxygen transport and storage, electron transport in aerobic respiration and signal transduction. Therefore, the synthesis of heme iron is vital. Heme synthesis occurs primarily in bone marrow, where red blood cells are synthesized, and in the liver to carry out its function in the cytochrome system. Of importance is that heme synthesis relies on vitamin B\textsubscript{6} and zinc, thus deficiencies in these nutrients negatively impact iron homeostasis.

1.2.2.3 Iron Toxicity

Chronic iron toxicity can be associated with high dietary iron intake, frequent blood transfusions, and hemochromatosis, a genetic disease associated
with increased iron absorption. However, there are mechanisms in place to prevent toxic accumulation of iron, including the upregulation of hepcidin to reduce iron absorption, induction of ferritin to limit the availability of iron and the induction of antioxidant enzymes for protection against oxidant damage. Therefore, the risk of iron toxicity is low.

1.2.3 Assessment of Iron Status

There are several methods of assessing iron status and diagnosing iron deficiency, and the precise cutoff values often differ by laboratory. The most specific test for depletion of iron storage is low serum ferritin. However, ferritin is an acute-phase protein and is thus increased in a state of chronic or acute inflammation. Therefore, this method is not always reliable since high ferritin may occur during conditions of low iron status. Serum iron, the amount of iron in the blood, is not representative of the amount of iron stored in the body, and therefore this test is rarely informative on its own. Transferrin is a protein that transports iron in blood via reversible binding. Total iron-binding capacity (TIBC) measures how much of transferrin in circulation is iron-free. Thus, elevated TIBC indicates poor iron status. Transferrin saturation index is the ratio of serum iron to TIBC and represents how much serum iron is bound to transferrin. Low transferrin saturation indicates iron deficiency. The soluble form of transferrin receptor present in serum (sTfR) is proportional to the amount of surface receptors; sTfR expression is translationally regulated and is increased when iron stores are low.
1.3 Anemia

The widely known clinical manifestation of iron deficiency is anemia. Anemia is generally characterized by low hemoglobin concentrations (<12 g/dL for menstruating females and <13 g/dL for men and post-menopausal females). The World Health Organization (WHO) estimates that 39% of children less than 5 years of age, 48% of 5-14 year-old children, and 42% of women in developing countries suffer from anemia, but iron deficiency is present in only half of these individuals. Anemia is also a consequence of deficiencies in other nutrients including copper, vitamins B\textsubscript{6} and B\textsubscript{12}, folate, and zinc, which adds to the complexity of effectively diagnosing and treating iron deficiency. Anemia resulting from iron, copper and vitamin B\textsubscript{6} deficiency is characterized as microcytic, while anemia due to deficiency in vitamin B\textsubscript{12} and folate are characterized as megaloblastic.

1.3.1 Microcytic Anemia: Iron, Copper and Vitamin B\textsubscript{6} Deficiency

1.3.1.1 Iron Deficiency Anemia (IDA)

Severe iron deficiency is accompanied by microcytic, hypochromic anemia and is termed IDA.\textsuperscript{36,37} IDA is characterized by the aforementioned measures of iron depletion, as well as low mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV), and high red blood cell width (RDW).\textsuperscript{15}
1.3.1.1 IDA Secondary to Copper Deficiency

IDA may also occur secondary to copper deficiency. Hephaestin is a copper-containing ferroxidase that oxidizes ferrous iron to ferric iron, enabling iron to bind transferrin and enter circulation. Therefore, copper deficiency may also lead to functional iron deficiency and IDA.

1.3.1.2 Vitamin B<sub>6</sub> and Zinc Deficiency

Vitamin B<sub>6</sub> and zinc are required for heme synthesis, and therefore deficiencies in these nutrients may result in anemia. Similar to iron deficiency anemia, vitamin B<sub>6</sub> (pyridoxine) deficiency can manifest as microcytic, hypochromic anemia (low MCH and low MCV), but with normal or high serum concentrations of iron and ferritin. This type of anemia is generally named sideroblastic anemia, referring to ringed sideroblasts in the bone marrow, and is thought to be caused by insufficient hematopoiesis due to faulty heme synthesis. Pyridoxine-responsive anemia belongs to this group, as pyridoxine acts as a coenzyme in the process of heme synthesis. Since zinc is also required for heme synthesis, zinc deficiency may also manifest as microcytic, hypochromic anemia; however, there is a lack of evidence to support this in the literature. Therefore, in addition to iron and copper, deficiency in vitamin B<sub>6</sub> and zinc may result in microcytic anemia.
1.3.2 Megaloblastic Anemia: Vitamin B\textsubscript{12} and Folate Deficiency

Vitamin B\textsubscript{12} and folate are important for the normal maturation of all cells, as they are needed for cell differentiation.\textsuperscript{45} Vitamin B\textsubscript{12} and folic acid deficiency are characterized by megaloblastic anemia, which affects the development and morphology of blood cells. The anemia of folic acid deficiency has morphologic features indistinguishable from those of vitamin B\textsubscript{12} deficiency, but it develops much more rapidly.\textsuperscript{15,46-48} The cellular hallmarks are macrocytosis, which is easily detected by elevated erythroid MCV but not specific to megaloblastic anemia, and abnormal nuclear maturation that is more specific than macrocytosis.\textsuperscript{15,45} However, normocytic anemia (normal MCV) may be attributed to vitamin B\textsubscript{12} and folate deficiency anemia in persons with coexisting microcytosis, possibly caused by iron deficiency anemia, which can blunt or eliminate the expected macrocytosis.\textsuperscript{49,50} Therefore, vitamin B\textsubscript{12} and folate can lead to anemia; however, this type of anemia is characterized by macrocytosis, and thus may be distinguishable from microcytic anemia caused by iron, copper, vitamin B\textsubscript{6} and zinc deficiency.

1.3.3 Anemia of Chronic Disease (ACD)

Anemia of Chronic Disease (or Anemia of Inflammation) may accompany chronic illness or inflammation, and is a protective mechanism that the body uses to limit the amount of iron that is available to harmful pathogens.\textsuperscript{51} When this defense system is activated, less iron is absorbed and excess iron is collected by macrophages and stored in the liver, resulting in a rise in ferritin and a decrease
in hemoglobin concentrations.\textsuperscript{51} There are three known mechanisms for ACD, which are all related to abnormal cytokine levels: 1) Dmt1 is upregulated and Fpn1 is downregulated by lipopolysaccharides, interferon-γ, and tumor necrosis factor-α (TNF-α), causing an increase in iron uptake by reticuloendothelial cells; 2) Hepcidin is upregulated by lipopolysaccharides, interleukin 6 (IL-6), and possible interleukin-1 (IL-1), causing a reduction in iron absorption; 3) Erythroid progenitor expansion is inhibited by interferon-γ, IL-1, TNF-α, and interferon-β, causing disruption of red blood cell production.\textsuperscript{52} Patients with chronic or inflammatory disease are identified with high serum C reactive protein (CRP), low hemoglobin concentrations and low transferrin saturation.\textsuperscript{15,51} Because patients who have IDA may also have co-existing inflammation, it is difficult to differentiate between ACD and IDA. Since serum ferritin concentrations may be increased in patients who are inflamed with IDA, it may not be an accurate measure. To differentiate between the diagnoses of ACD with and without iron deficiency, TIBC and sTfR are useful. TIBC is low in ACD, unlike IDA, because there is ample iron but it is not readily available.\textsuperscript{51} Additionally, a derived value for the ratio of sTfR to log ferritin greater than 2 defines ACD with iron deficiency, whereas a ratio of sTfR to log ferritin less than 1 is typical in ACD without iron deficiency.\textsuperscript{53} Therefore, anemia may also be associated with conditions that are unrelated to micronutrient status.
1.4 Iron Deficiency in Obesity

Although iron deficiency is generally associated with under-nutrition, overweight individuals are also at a higher risk of iron deficiency than normal-weight individuals. Several studies have shown an inverse relationship between an individual’s BMI and iron status.54-56

1.4.1 Mechanisms

Potential mechanisms for impaired iron status in the obese state include decreased dietary intake of iron and increased intake of dietary fat, impaired intestinal uptake and release of iron, and reduced iron bioavailability due to inflammation. Through these mechanisms, obesity can influence both true and functional iron deficiency. To add to the complexity, it is difficult to diagnose iron deficiency in the setting of obesity due to co-existing inflammation, which increases the release of acute-phase reactants such as ferritin and thus iron status may appear artificially high, even in a state of true iron deficiency.57

1.4.1.1 Changes in Dietary Intake

1.4.1.1.1 Dietary Iron Intake

Low dietary intake of iron and high intake of other nutrients in obesity may contribute to iron deficiency. In a cross-sectional study of 512 adolescents, both overweight boys and girls had lower iron intakes than their non-overweight counterparts.58 However, other studies have not confirmed this finding. For example, in a cross-sectional study of 207 obese and 178 non-obese adults, iron
deficiency was significantly more prevalent, mean serum iron was lower, and transferrin saturation was lower among obese compared with non-obese subjects, despite no differences in dietary iron intake between these groups. In a cross-sectional survey, the risk of iron deficiency in obese Mexican women and children was 2-4 times that of normal-weight individuals even though dietary iron intakes were similar. Therefore, it is not conclusive that low iron intake is the reason for poor iron status in obesity.

1.4.1.1.2 Dietary Fat Intake

Another study investigated the effect of high-fat-diet-induced obesity on iron metabolism in an animal model of diet-induced obesity. C57BL/6 mice were placed on a high fat content diet (HFD) or a standard diet (SD) with high carbohydrate content for eight weeks, and then further randomized to receive or not receive iron supplementation for an additional two weeks. Mice receiving the HFD had significantly higher body weight when compared with SD-fed C57BL/6 mice (P< 0.001). HFD feeding decreased serum and hepatic iron levels, and response to iron supplementation. Duodenal absorption of ferric citrate was significantly decreased in HFD-fed mice compared to SD-fed mice 60 and 90 minutes after gavage (P<0.05). The authors further investigated the underlying mechanisms of reduced iron absorption in the HFD group by measuring the mRNA expression of duodenal oxidoreductases, and found that Dcytb and hephaestin expression were decreased with HFD compared to SD
with or without iron supplementation. This data suggests that interaction between dietary iron and excess fat in the intestinal lumen may reduce iron absorption by reducing iron uptake and extrusion by oxidoreductases Dcytb and hephaestin, respectively.

A human pilot study suggested that the type of dietary fat may also affect iron absorption. Three male cyclists consumed isoenergetic, isonitrogenous diets for 28-day periods in a randomized, crossover design in which dietary carbohydrate, polyunsaturated (PUFA), or saturated fat contributed to roughly 50% of daily energy intake. Fecal iron increased and iron balance (intake – loss) decreased with the diet high in polyunsaturated fat. Similarly, fecal iron increased and iron balance decreased with an increase in linoleate intake. Since fecal iron is an indirect measure of iron absorption, this study suggests that iron absorption decreases with a diet high in PUFA, particularly linoleic acid. However, a diet high in saturated fat did not have the same effect. Therefore, larger clinical studies are needed to determine a relationship between consumption of specific dietary fats and iron absorption.

1.4.1.2 Obesity-induced Inflammation

Obesity induces low-grade, chronic, systemic inflammation that may contribute to reduced iron absorption and bioavailability, resulting in true and functional iron deficiency. This is illustrated by a clinical study of 92 premenopausal Thai women in which fractional iron absorption was negatively
correlated with CRP (p<0.001) and BMI (P<0.05) independent of serum ferritin levels, and CRP was significantly positively correlated with BMI. This data suggests that adiposity-induced inflammation is associated with lower fractional iron absorption in humans. The authors hypothesized that inflammation-induced hepcidin was likely causing a decrease in dietary iron absorption by reducing Fpn1 expression, known as the “Hepcidin Mechanism.”

1.4.1.2.1 Hepcidin Mechanism

Several authors speculate that increased circulating hepcidin in obesity may be a contributing factor leading to the reduced iron status observed in the obese population. In support of this claim, both cell culture and human studies suggest that hepcidin levels are increased in obesity. The inflammatory adipokine, leptin, which is secreted by adipose tissue and circulates at levels proportional to body fat, promoted hepcidin expression in HuH7 human hepatoma cells in a dose-dependent manner. In a human study of 105 candidates for bariatric surgery and 60 control subjects who were stratified according to BMI category, moderate and severe obesity (BMI > 35 kg/m²) were significantly associated with higher serum hepcidin concentration. These data support the notion of increased hepcidin levels in obesity. Furthermore, in a study of 60 obese children and 50 control subjects, a direct correlation between hepcidin and obesity degree (P=0.0015) were observed, as well as inverse correlations between hepcidin and transferrin saturation (P=0.005) and iron
absorption (P=0.003). This serves as evidence for the link between obesity, hepcidin, and impairment of iron status.

Another study explored the tissue-specific role of hepcidin in iron regulation. This study included three groups of obese patients (obese, obese with diabetes, and obese with nonalcoholic steatohepatitis [NASH]), control adipose tissue from lean females, and control liver samples which were histologically normal and non-inflamed from males and females. These patients were investigated to determine the possible role of hepcidin in severe obesity. Low transferrin saturation was observed in 68% of the patients and 24% of these patients presented with anemia. Hepcidin mRNA and protein levels in subcutaneous adipose tissue (SAT) were significantly enhanced in all obese conditions compared to control adipose tissue samples, and SAT hepcidin mRNA expression was correlated with the BMI of the patients; there was no effect of diabetes or NASH on hepcidin expression levels in liver or adipose tissue. Also, the hepcidin mRNA concentration in adipose tissue was related to the expression of inflammatory markers CRP and IL-6. However, hepcidin expression in adipose tissue was similar in iron-depleted and iron non-depleted obese groups, suggesting that adipose-derived hepcidin may play a minimal role in iron mobilization in obesity. Interestingly, liver biopsy showed that hepatic hepcidin mRNA expression levels in obese individuals were not significantly different from those of control liver samples. Still, there was a negative correlation found between liver, but not adipose tissue, hepcidin expression and serum transferrin saturation in the obese, suggesting that the effects of hepcidin may be tissue
specific.\textsuperscript{65} Thus, further studies are needed to determine the role of adipose versus hepatic production of hepcidin and adverse effects on iron homeostasis in obesity.\textsuperscript{65}

1.4.1.2.2 Hepcidin-Independent Mechanism

Inflammation, through mediators that are separate from hepcidin production, may induce iron sequestration in adipose tissue and liver, thereby contributing to iron deficiency in obesity. mRNA expression and circulating concentrations of lipocalin-2 in adipose and hepatic tissue were significantly increased in db/db leptin receptor deficient, obese mice compared with their lean littermates (P<0.001).\textsuperscript{30,66} Lipocalin-2 is an iron-binding protein that is upregulated by inflammation and may promote sequestration of iron during infections to limit bacterial growth.\textsuperscript{29,62} Therefore, inflammation may induce functional iron deficiency by adipose and hepatic sequestration of iron.

1.4.1.3 Summary of Mechanisms Responsible for Iron Deficiency in Obesity

In summary, obesity may increase risk for iron deficiency by 1) reducing iron absorption in the intestine due to high consumption of fat, 2) decreasing iron mobilization from the intestine and peripheral tissue via hepcidin which is induced by inflammation, and 3) increasing the sequestration of iron in adipose and hepatic tissues via inflammation-induced upregulation of lipocalin-2.
1.5 Bariatric Surgery for the Treatment of Obesity

Bariatric surgery is growing in popularity as a treatment to induce weight loss in patients who are moderately (body mass index, BMI ≥ 35 kg/m$^2$) and severely (BMI ≥ 40 kg/m$^2$) obese. The most recent 2013 guidelines for the management of overweight and obesity in adults, endorsed by the American Heart Association, the American College of Cardiology, and The Obesity Society, strongly recommend that adults with a BMI ≥ 40 or BMI ≥ 35 with obesity-related comorbid conditions, who are motivated to lose weight and have not responded to behavioral treatment, be referred to an experienced bariatric surgeon for evaluation.$^{67}$ In addition, bariatric surgery has been implicated in the amelioration of comorbid cardiometabolic diseases.$^{13,68-73}$ Bariatric surgery may also decrease risk of mechanical (arthritis and osteoarthritis, respiratory diseases and sleep apnea) and psychosocial (depression and eating disorders) complications of obesity.

Bariatric surgery induces weight loss by altering the gastrointestinal tract to promote food restriction and/or malabsorption. Therefore, bariatric surgery promotes deficiencies in various micronutrients including iron.$^{68}$ As a result, obese patients who choose to undergo bariatric surgery may experience the ‘double burden’ of iron deficiency caused by pre-existing obesity, as well as changes in dietary intake, nutrient absorption, and inflammation following bariatric surgery. The risk of iron deficiency varies with each type of surgery and is related to the anatomical alterations that are made. The anatomical descriptions of the various types of bariatric surgery have been extensively
reviewed and are shown in figure 1.2. The following is a brief overview of the surgeries.

Figure 1.2 Anatomies of Bariatric Procedures. (A) Laparoscopic adjustable gastric banding (LAGB). (B) Roux-en-Y gastric bypass (RYGB). (C) Sleeve gastrectomy (SG). (D) Biliopancreatic diversion with duodenal switch (BPD-DS).
1.5.1 Restrictive Bariatric Surgeries

Restrictive procedures achieve weight loss by reducing stomach volume or absorptive capacity. The two currently-performed restrictive procedures are the adjustable gastric banding (AGB) and sleeve gastrectomy (SG) procedures. Recent 2011 data show that the former is decreasing, while the latter is growing, in popularity.74

1.5.1.1 Adjustable Gastric Banding (AGB)

In the AGB procedure (figure 1.2a), a band is placed around the proximal stomach, encompassing the upper portion of the fundus and creating a temporary holding pouch of 30 mL. When food is ingested, the pouch expands and creates intraluminal pressure, which triggers satiety signals.75 This surgery requires that a trained medical professional periodically tighten the band around the stomach to maintain the feeling of fullness. Routine band adjustment largely determines the effectiveness of this procedure. If the flow of food is obstructed by the narrowness of the band, nutritional complications, such as reflux, nausea, and/or vomiting may occur. AGB is associated with reduced tolerance for course-textured foods, which may lead to the avoidance of red meats and fibrous fruits and vegetables.76-78

1.5.1.2 Sleeve Gastrectomy (SG)

In the SG procedure (figure 1.2c), the larger curvature of the stomach is dissected, leaving a narrow vertical tube that can hold a volume of 150 mL.
Similar to AGB, SG achieves earlier satiety following a meal.\textsuperscript{68,79} In addition to reducing stomach volume, increased satiety may occur by decreases in the appetitive hormone ghrelin and increases in the satiety hormone polypeptide YY that have been observed following SG.\textsuperscript{80} Also, SG may decrease gastric emptying half-time, which is the time required by the stomach to empty 50% of the ingested meal, as well as small bowel transit time,\textsuperscript{81} thus limiting the digestion, absorption, and bioavailability of some nutrients.\textsuperscript{68}

1.5.2 Combined Restrictive and Malabsorptive Bariatric Procedures

Malabsorptive procedures alter the flow of food to limit contact with pancreatic secretions and/or bypass absorptive regions of the intestine in order to limit nutrient absorption.\textsuperscript{68}

1.5.2.1 Roux-en-Y Gastric Bypass (RYGB)

The roux-en-y gastric bypass (RYGB, \textit{figure 1.2b}) procedure is the most common procedure in the United States\textsuperscript{82} and combines restrictive and malabsorptive mechanisms. In the RYGB procedure, the stomach is reduced to a volume of 20-30 mL, and the food bolus is rerouted to the distal jejunum via an anastomosis connection.\textsuperscript{83} Although malabsorption of proteins and fats occurs at least initially after RYGB, restricted dietary intake plays a much larger role in the reduction of energy intake.\textsuperscript{84} Weight loss associated with alterations in the secretion of appetitive and satiety gastrointestinal hormones may be even more
pronounced in RYGB compared to SG, as RYGB induces decreased fasting ghrelin concentrations, as well as increased postprandial PYY and glucagon-like peptide 1 (satiety hormone) concentrations. In addition, shunting of undigested food, particularly if high in simple carbohydrates or fat, directly to the lower jejunum, which serves as the common channel, can produce an osmotic imbalance that triggers severe gastrointestinal symptoms such as bloating, nausea, vomiting, flatulence, and diarrhea, collectively known as dumping syndrome. Thus, following RYGB, patients develop food intolerances and avoid nutrient-dense foods such as meat, milk, and foods high in fiber.

1.5.2.2 Biliopancreatic Diversion (BPD)

The biliopancreatic diversion (BPD) and the more recent version, the biliopancreatic diversion with duodenal switch (BPD-DS, figure 1.2d), are primarily malabsorptive procedures, although their popularity in the United States and other countries is low. In the BPD-DS, the stomach is resected as is done for the SG, and then the intestine is divided at the duodenum after the pyloric sphincter in the proximal small intestine, and again in the distal intestine, creating biliopancreatic and alimentary limbs, respectively. The alimentary limb is brought up and connected to the duodenum, causing food leaving the stomach to bypass most of the small intestine. Thus, immediately following exit from the stomach, the food bolus is diverted and then rejoined with the biliary and pancreatic juices in a common channel that begins at the ileum. This procedure achieves greater
weight loss than RYGB, but is associated with more early complications and slightly higher perioperative mortality.\textsuperscript{87}

1.5.3 Consequences of Bariatric Surgery

Poor nutritional outcomes have been associated with all weight loss surgeries. These may include macro- and micronutrient deficiencies as well as weight regain.\textsuperscript{68} In patients who experience weight regain, chronic obesity-related inflammation will likely persist following surgery. Notably, in the years following surgery the intestine adapts to improve macronutrient absorption,\textsuperscript{88} however the effect of gut adaptation on micronutrients has not been clearly demonstrated. Nutrient deficiencies may be a result of decreased dietary intake or retention of food following surgery, reduced absorption, or decreased bioavailability.

1.5.3.1 Iron Deficiency

Iron deficiency has been well-documented following bariatric surgery (postoperative prevalence: 15-54\%\textsuperscript{8-12}), but it is difficult to diagnose and treat due to its multifactorial etiology. This is partly because deficiencies in other nutrients may co-exist with deficiency in iron following surgery, and also that the signs and symptoms of iron deficiency, such as anemia, are shared with other nutrient deficiencies.\textsuperscript{11}

Of the common bariatric surgeries, the frequency of iron deficiency is highest in RYGB.\textsuperscript{89-92} In a large cohort study (N=21,345), the prevalence of iron
deficiency in RYGB patients steadily increased from the pre-bariatric surgical period through the post-bariatric surgical periods; the prevalence was 5% pre-surgery, 10% at 0-12 months, 13% at 13-24 months, and 21% at 25-36 months.\cite{93} Similarly, clinical records of 30 patients who underwent laparoscopic RYGB surgery between July 2003 and January 2005 showed that the number of patients who presented with iron deficiency increased from 6.6% before surgery to 40% at 2 years and 54.5% at 3 years following surgery.\cite{11} These findings are consistent with others that show exacerbation of iron deficiency following RYGB.\cite{8,10,11,68,94-97} The range in estimates may be attributed to differences in the indices used, the period of time evaluated post-surgery, and the occurrence of iron deficiency without symptoms.\cite{68} However, symptomatic manifestations of iron deficiency after RYGB include hematological disorders such as chronic anemia and even pica, a condition associated with iron deficiency inducing unusual cravings for ice, cornstarch, clay, and other substances.\cite{89} Premenopausal women who undergo RYGB have an increased risk of iron deficiency due to regular blood loss during menses.\cite{68} In order to prevent iron deficiency and its disabling symptoms following surgery, it is important to elucidate the mechanisms by which iron status is reduced.

1.6 Mechanisms for Iron Deficiency following Bariatric Surgery

Changes in dietary intake, absorption, and bioavailability, due to mechanical alterations that are inherent to each surgical procedure, contribute to iron deficiency following bariatric surgery.
1.6.1 Changes in Dietary Intake

1.6.1.1 Iron intake

Dietary intake of iron was reported to be lower than the recommended amount following sleeve gastrectomy and roux-en-y gastric bypass surgeries.\textsuperscript{68,94,98} Decreased iron intake and retention may be related to gastrointestinal distress, including intolerance of iron-containing foods, as well as reduced availability of other nutrients whose levels are also decreased post-surgery. Some evidence suggests that intake of meat, a good source of bioavailable heme iron, is reduced, which contributes to iron deficiency after bypass surgery.\textsuperscript{68,89} In one study, 27 out of 69 patients who had received gastric bypass experienced emesis (vomiting) after consuming fibrous meats.\textsuperscript{99} In another study, 50\% of bypass patients experienced chronic meat intolerance after surgery.\textsuperscript{100} However, in a randomized control trial comparing bypass with banded patients, only the bypass patients were iron deficient, despite having better tolerance for eating red meat.\textsuperscript{91} Therefore, low meat intake alone may not explain iron deficiency in bariatric surgery patients.

1.6.1.2 Intake of Nutrients Related to Iron

Dietary intake of other nutrients, such as copper, is also important as these nutrients may enhance or inhibit iron absorption and metabolism. Copper is a component of the multicopper ferroxidase hephaestin, which catalyzes the
conversion of Fe$^{2+}$ to Fe$^{3+}$ so that it can be transported by transferrin into circulation. Notably, copper deficiency following bariatric surgery has been reported to have a prevalence ranging from 10 to 15% and an incidence ranging from 4 to 18%. Copper deficiency following surgery may result from insufficient copper in micronutrient supplementation and malabsorption of copper in the duodenum and proximal jejunum. Therefore, malnutrition in copper may contribute to iron deficiency in bariatric surgery patients.

1.6.2 Reduced Iron Absorption

Mechanisms related to anatomical alterations following bariatric surgery may contribute to reduced iron absorption and bioavailability. Heme and non-heme iron absorption are reduced after SG and RYGB.

1.6.2.1 Diminished Gastric Acid Secretion

Diminished gastric acid secretion and exclusion of the duodenum may contribute to iron malabsorption. In malabsorptive procedures the antrum is separated from the proximal gastric pouch, resulting in diminished gastric acid secretion. A longitudinal study showed a significant post-surgical decrease in acid secretion in eight patients who underwent gastric bypass. Reduced acid secretion hinders the reduction of ferric iron and ultimately absorption of ferrous iron in the duodenum. In support of this mechanism, banding procedures, which
maintain gastrointestinal continuity, have lower rates of iron deficiency than bypass procedures.\textsuperscript{89,104,105}

1.6.2.2 Duodenal Exclusion

Excluding the duodenum from digestive continuity may also contribute to decreased iron absorption. Most absorption of non-heme and highly bioavailable heme iron occurs across duodenal enterocytes.\textsuperscript{28} Therefore, it is not surprising that decreasing the amount of duodenal absorptive surface area may diminish iron absorption following malabsorptive procedures. In support of this point, patients undergoing BPD-DS, which preserves some proximal duodenal function compared to BPD, may experience less iron deficiency.\textsuperscript{89} BPD-DS patients had significantly higher serum ferritin levels than BPD patients.\textsuperscript{89,106} However, a recent study comparing BPD and BPD-DS found no significant difference in serum iron levels.\textsuperscript{89,107} Additional information is needed to draw conclusions because serum iron is not a good indicator of iron status.

1.6.2.3 Decreased Duodenal Absorptive Capacity

In addition to decreasing duodenal absorptive capacity, malabsorptive procedures delay interaction between pancreatic enzymes and biliary secretions and the food bolus until the common channel. This has a pronounced adverse effect on heme iron absorption, as biliopancreatic secretions are necessary for proteolytic digestion of myoglobin and hemoglobin, and ultimately the release of
heme that is available for absorption.\textsuperscript{89,108} Therefore, incomplete digestion of heme iron may exacerbate iron malabsorption.

1.6.3 Inflammation

1.6.3.1 Bacterial Overgrowth

Inflammation caused by bacterial overgrowth may reduce iron bioavailability via mechanisms similar to obesity-related inflammation. Malabsorptive bariatric surgery may induce bacterial overgrowth and inflammation in the functional and remnant stomach.\textsuperscript{82} In a prospective observational study of 35 RYGB patients who had predominantly normal gastric endoscopic findings before surgery, 74.3\% developed various types of gastritis, or inflamed lining of the stomach, almost exclusively in the excluded stomach.\textsuperscript{109} Also, in post-RYGB subjects, high microbial counts were detected in 59.5\% of samples from the functional gastric pouch and 18.9\% of samples from the excluded reservoir.\textsuperscript{110} Infection by \textit{Helicobacter pylori} was correlated with bacterial overgrowth.\textsuperscript{110} Therefore, inflammation due to bacterial overgrowth may contribute to iron deficiency following bariatric surgery.

1.6.3.2 The Inflammation Discrepancy

A paradoxical impact of bariatric surgery is that although it appears to impair iron status, it also promotes long-term weight loss, thereby ameliorating chronic inflammation of obesity, and thus may improve iron status over time.
Following bariatric surgery, reductions in BMI, CRP, IL-6, and orosomucoid are well-documented.\textsuperscript{111,112,113} One study showed a significant increase in transferrin saturation at six months following surgery, which the authors attributed to the reduction of inflammation, as CRP and orosomucoid were inversely correlated with transferrin saturation before and after surgery.\textsuperscript{113} This data suggests that bariatric surgery-induced weight loss is associated with reduction of inflammation and improvement in functional iron status in these patients. However, this finding is in contrast to the findings of several studies that show exacerbation of iron deficiency up to three years following RYGB.\textsuperscript{11,93} Therefore, there may be other factors that may explain the discrepancy, such as the time that has elapsed since surgery.

1.7 Research Questions

With the increasing trend of severe obesity, there is an increase in the number of bariatric procedures performed worldwide.\textsuperscript{114} RYGB is the most commonly performed bariatric procedure.\textsuperscript{114} A high prevalence of iron deficiency is documented following RYGB (10-45\%).\textsuperscript{8,10,11,94-97} Hemoglobin concentrations were significantly lower in patients receiving gastric bypass than in restrictive bariatric surgeries\textsuperscript{115} and prevalence of anemia as high as 64\% has been reported following RYGB.\textsuperscript{11,96,115-119} A well-established mechanism for iron deficiency following RYGB shown by Ruz et al is reduced iron absorption.\textsuperscript{101,102} Other suggested mechanisms include reduced intake of iron and other nutrients related to iron homeostasis, and persisting obesity-induced or other sources of
inflammation. Chapter 2 describes studies in which we document the high prevalence of iron deficiency and the associated clinical manifestation of anemia following RYGB as well as explore some mechanisms responsible. Our overall objective was to describe iron homeostasis following RYGB by using comprehensive screening approach to assess micronutrient status, dietary intake, and anemia in RYGB patients. The first aim was to conduct a comprehensive screening of nutrients involved in iron homeostasis in RYGB patients. We hypothesized that iron deficiency occurs in combination with other nutrient deficiencies. The second aim was to determine the contribution of dietary intake to the micronutrient profile of RYGB patients. We hypothesized that low intake of iron and intake of nutrients involved in iron homeostasis from diet and supplements contributes to malnutrition in iron following RYGB. The third aim was to describe associations between anemia and nutritional status in RYGB patients. We hypothesized that anemia is associated with iron deficiency as well as deficiencies in other nutrients following RYGB.
CHAPTER 2. COMPREHENSIVE ASSESSMENT OF IRON NUTRITUDE IN ROUX-EN-Y GASTRIC BYPASS SURGERY PATIENTS

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2.2 Abstract

Roux-en-Y gastric bypass surgery (RYGB) is effective for weight loss, but is commonly associated with iron deficiency and its clinical manifestation, anemia. Diagnosing iron deficiency is complex because iron status depends on other nutrients; additionally, anemia following surgery is not specifically due to deficiency in iron, as it can be due to deficiencies in other nutrients including zinc, copper, vitamin B₆, folate, and vitamin B₁₂. In patients who have undergone RYGB, our aims were to 1) conduct a comprehensive assessment of nutrients involved in iron homeostasis, 2) determine the contribution of dietary intake to iron deficiency, and 3) describe associations between anemia and nutritional status of iron and other nutrients. Systemic measures of hemoglobin, ferritin, serum transferrin receptor (sTfR), total iron binding capacity (TIBC), copper (Cu), vitamins B₆ and B₁₂, folate, zinc (Zn), and C-reactive protein (CRP) were determined using reference methods. Iron deficiency equaled having ≥ 2 abnormalities in: ferritin, sTfR, sTfR:ferritin, or TIBC. Ferritin, a measure of iron stores, was defined as normal (ferritin ≥ 20 mcg/L) or low (ferritin < 20 mcg/L). Statistics included prevalence, mean ± standard error of the mean (s.e.m.) for normally-distributed data, median ± semi-interquartile range for skewed data (indicated with an asterisk [*]), frequency tables, t-tests (independent, by group), correlations, and general linear models (significant if p < 0.05). Subjects (N=70) were 91% female, age 49 ± 1 years, *4 ± 2 years post surgery, and 79% Caucasian. Fifty-six percent of the total population and 96% of the subpopulation with anemia (N=26) presented with deficiencies related to iron nutriture, including
deficiencies in iron, zinc, copper, vitamin B₆, folate, and vitamin B₁₂. The most prevalent nutrient deficiencies in the total population and the subpopulation with anemia were iron and zinc; prevalence of iron and zinc deficiency in the total population was 24.3% and 20.0%, respectively, and prevalence of iron and zinc deficiency in the subpopulation with anemia was 46.2% and 23.1%, respectively. Participants in the total population and the subpopulation with anemia were also deficient in copper, vitamin B₆, vitamin B₁₂, and folate (11.5% and 26.9%, respectively). In the total population, iron and zinc deficiency occurred in isolation and also in combination with other nutrient deficiencies; all other nutrient deficiencies occurred in combination. In the subpopulation with anemia, only iron deficiency occurred in isolation. The dietary intake of the study population exceeded the RDAs for all nutrients assessed. In addition, patients with low ferritin concentrations consumed lower total energy (p= 0.009), fat (p= 0.026), protein (p=0.013), and animal protein (p=0.023), compared to patients with normal ferritin concentrations. Dietary intake of heme iron was correlated with years post-RYGB surgery (r=0.67, p<0.05). In conclusion, in a community-based surveillance of RYGB patients we found that, more often than not, RYGB patients presented with micronutrient deficiencies related to iron nutriture; this includes deficiencies in iron, copper, zinc, vitamin B₆, folate, and vitamin B₁₂. It was more likely for multiple deficiencies to occur simultaneously than for deficiencies to occur alone. In addition, there was a high prevalence of anemia, a clinical manifestation of deficiencies in these nutrients. Implications of these findings are that clinicians who evaluate post-RYGB patients, especially patients who present
with known symptoms or manifestations of iron deficiency, should also screen patients for deficiencies in copper, zinc, vitamin B₆, folate, and vitamin B₁₂, as these deficiencies are also prevalent following surgery, may occur simultaneously with iron deficiency, and are also implicated in anemia. Based on our findings that RYGB patients who have more favorable iron stores consume more energy, fat, and protein than patients with low iron stores, increasing protein intake following surgery may improve iron status; although, increasing consumption of fat may have detrimental effects on weight regain in the RYGB population. Findings of this study may be used to enhance prophylactic measures and treatments for iron deficiency following RYGB.

2.3 Introduction

The rate of obesity (BMI > 30 kg/m²) is increasing; in particular, there is an increasing trend in the severely obese category (BMI ≥ 40 kg/m²).² Bariatric surgery is an effective treatment for weight loss, as well as the improvement or resolution of obesity-related comorbidities including diabetes, hyperlipidemia, hypertension, and obstructive sleep apnea.¹²⁰ Therefore, it is not surprising that as the number of severely obese persons increases, there is a parallel increase in the number of bariatric procedures performed worldwide.¹¹⁴ The roux-en-y gastric bypass (RYGB) procedure is the most commonly performed bariatric procedure in the United States and combines restrictive and malabsorptive mechanisms that promote weight loss. In the RYGB procedure, the stomach is reduced to a volume of 20-30 mL, and the food bolus is rerouted to the distal
jejunum via an anastomosis connection; therefore, patients who undergo RYGB are vulnerable to nutrient deficiencies due to restricted food intake and malabsorption of nutrients.

There is a high prevalence of iron deficiency in obese individuals who are candidates for bariatric surgery (28%-35%) and following RYGB (10-45%). Iron deficiency is the most common and widespread nutritional disorder in the world, and the only nutrient deficiency that is significantly prevalent in industrialized nations. Iron deficiency is associated with increased infant mortality and perinatal risks for mothers and neonates, and adversely affects cognitive performance, behavior, and physical growth of infants and adolescents. Iron deficiency also impairs immune status, increases morbidity from infections, and reduces physical capacity and work performance in all age groups. Severe iron deficiency is associated with low blood concentrations of hemoglobin, and is termed iron deficiency anemia (IDA). Reported mechanisms that may contribute to the high prevalence of iron deficiency following RYGB include reduced heme and non-heme iron absorption and reduced dietary iron intake.

Assessment of iron status is complex. First, iron status depends on other nutrients, such as copper- which is involved in iron absorption via the enzyme hephaestin, vitamin B₆ and zinc- which are involved in heme synthesis, and vitamin B₁₂ and folate- which are needed for blood cell differentiation. Second, it is possible that deficiencies in multiple nutrients which impact iron status may occur simultaneously in patients who have undergone gastric bypass, although
there is a lack of evidence in the literature. Finally, the widely known clinical manifestation of iron deficiency, anemia (low hemoglobin), adds to the complexity of diagnosing iron deficiency. Anemia is observed after RYGB with a prevalence as high as 64%.\textsuperscript{11,94,113-117} However, anemia following surgery is not specific to deficiency in iron alone, as it can be due to deficiencies in other nutrients including copper, folate, vitamin B\textsubscript{12}, zinc, and vitamin B\textsubscript{6}.

To our knowledge, no one has explored the reasons for and consequences of the poor iron homeostasis observed following RYGB by describing micronutrient status, dietary intake, and anemia associated with the various nutrients implicated in iron nutriture. In patients who have undergone RYGB, our aims were to 1) conduct a comprehensive assessment of nutrients involved in iron homeostasis, 2) determine the contribution of dietary intake to iron deficiency, and 3) describe associations between anemia and nutritional status of iron and other nutrients. Findings of this study will be significant as they may be used to enhance prophylactic measures and treatments for iron deficiency following RYGB.

2.4 Patients and Methods

We used a marketing database to obtain addresses in the Greater Lafayette, IN community and recruited participants by mailing information about the study. Recruiting participants from the community and randomly selecting addresses afforded us a representative sample of bariatric surgery patients who
obtained their surgical procedures at various clinics and who had various lengths of elapsed time since surgery.

2.4.1 Study Population

The study began May of 2012. During recruitment, participants were pre-screened by telephone. They were eligible for screening if they underwent RYGB at least 6 months prior, and were 18-65 years of age. After August 2013, we added the additional criterion that participants must be currently experiencing anemia, and we also began to administer a three-day food record to each eligible participant to complete prior to the screening visit.

2.4.2 Metabolic Measures

We did a comprehensive screening of nutrients related to iron homeostasis. Plasma samples were obtained at screening and stored at -80°C. Ceruloplasmin activity and serum transferrin receptor were measured in-house. Ceruloplasmin activity was measured using the oxidase method.\textsuperscript{121} The coefficients of variation for the intra- and inter-assay variability were 3.6% and 3.8%, respectively. Soluble serum transferrin receptor was measured using the enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). Copper, ferritin, zinc, vitamins B\textsubscript{1}, B\textsubscript{6}, B\textsubscript{12}, folate, hemoglobin, TIBC, and C-reactive protein were commercially measured by Mid America Clinical Laboratories (MACL), Indianapolis, IN, USA. Serum copper and zinc were measured using inductively coupled plasma mass spectrometry (ICP/MS).
Vitamin B\textsubscript{1} from whole blood and serum vitamin B\textsubscript{6} were measured using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Serum ferritin was measured using a Chemiluminescent Microparticle Immunoassay (CMIA). Serum vitamin B\textsubscript{12} and folate were measured using chemiluminescence. Siemens Advia 2120i instrumentation was used by MACL to perform hemoglobin analysis. TIBC was measured using the tripyridyltriazine/ferine method. CRP was measured using the turbidimetric latex method.

Subjects were found to be deficient in iron if they met at least two of the following four conditions: TIBC > 370 mcg/dL (cutoff determined by MACL), serum ferritin < 20 mcg/L\textsuperscript{122,123}, serum transferrin receptor (sTfR) > 28.1 nmol/L, sTfR:ferritin ratio > 500\textsuperscript{122}. Subjects were found to be anemic if hemoglobin < 12 g/dL in menstruating women and hemoglobin < 13 g/dL in men and post-menopausal women.\textsuperscript{13} Deficiencies in other nutrients were defined as copper (serum copper < 70 mcg/dL determined by MACL; ceruloplasmin < 62.5 U/L\textsuperscript{121}), zinc < 60 mcg/dL, vitamin B\textsubscript{6} < 2.1 ng/mL, folate <5.4 ng/mL and vitamin B\textsubscript{12} < 220 pg/mL. Inflammation, measured by cardio C reactive protein (CRP), was abnormally high if > 3.1 mg/L (cutoffs determined by MACL).

2.4.3 Dietary Analysis

Nine participants completed three-day food records, for which we performed dietary analysis using Nutrition Data System for Research (NDSR); software (University of Minnesota, Minneapolis, MN, USA). The daily intake of macro- and various micronutrients was determined (Table 2.3). Dietary heme
iron was calculated by multiplying the number 0.4 by the total iron content of all meat items.

2.4.4 Statistical Analysis

The statistical software STATISTICA (StatSoft Inc., Tulsa, OK, USA) was used for analysis. Descriptive statistics were assessed using parametric tests and expressed as mean ± standard error of the mean (s.e.m.) for normally distributed data and median ± semi-interquartile range for skewed data. Values presented as median ± semi-interquartile range are indicated with an asterisk (*). Prevalence of micronutrient deficiencies were determined and defined as the number of cases of each micronutrient deficiency; the denominator was 70 subjects for the total population, 26 subjects for the population with anemia, and nine subjects for the population with dietary records. Differences between categorical groups were determined using independent t-tests; associations between nutrient status and continuous variables (such as dietary intake and blood concentrations of micronutrients) were determined using independent t-tests, by group. Pearson correlations between continuous variables were performed. We had the capability of using general linear models to determine potential interactions between covariates if we found significant correlations between variables; however, we found no correlations between variables of interest. Differences in prevalence of deficiency between populations were determined using chi-square analysis. Significance was defined as p < 0.05.
2.5 Results

2.5.1 Population Characteristics

*Table 2.1* shows the population characteristics for the total population, the subpopulation with anemia, and the subpopulation with completed three-day food records. In the total population, 70 eligible patients were assessed. The population was 91% female, 79% Caucasian and 14% African American. At the time of screening, subjects were 49.4 ± 0.9 years of age and were *4 ± 2* years post-RYGB surgery. The average BMI in the total population was *28.9 ± 2.7* kg/m². Fifty-one percent of the subjects in the total population reported taking multivitamin/multimineral supplements and 10% reported not taking these supplements; thirty-nine percent of the total population did not provide this information.

The subpopulation with anemia included 26 RYGB patients. The population was 100% female, 75% Caucasian and 25% African American. At the time of screening, subjects were 50.3 ± 1.2 years of age and were *4 ± 2* years post-RYGB surgery. The average BMI in the population with anemia was *26.4 ± 2.5* kg/m². Fifty percent of the subjects in the population with anemia reported taking multivitamins and 12% reported not taking multivitamins; thirty-eight percent of the subpopulation with anemia did not provide this information.

The subpopulation with three-day food records included nine RYGB patients. The population was 89% female and 100% Caucasian. At the time of screening, subjects were 48.0 ± 2.6 years of age and 5.4 ± 1.7 years post-RYGB surgery. The average BMI in the population with dietary records was *26.5 ± 2.7* kg/m².
kg/m². Seventy-eight percent of the subjects in the population with dietary records reported taking multivitamins and 22% reported not taking multivitamins.

2.5.2 Nutrient Deficiencies

Table 2.2 shows the prevalence of deficiency for nutrients involved in iron homeostasis in the total population, the subpopulation with anemia, and the subpopulation with dietary records. In 55.7% of the total population, we found deficiencies in nutrients related to iron nutriture. Prevalence of deficiency in the total population for iron (Fe), zinc (Zn), copper (Cu), vitamin B₆ (B₆), folate (Fol), and vitamin B₁₂ (B₁₂) from highest to lowest were: Fe= 24.3%, Zn= 20.0%, B₆= 4.3%, Cu= 2.9%, B₁₂= 2.9% and Fol= 1.4%. In 44.3% of the total population we did not find deficiencies related to iron homeostasis.

Prevalence of anemia in the total population was 37.1%. In 96.2% of the subpopulation with anemia, we found deficiencies in nutrients related to iron nutriture. Prevalence of deficiencies in the subpopulation with anemia from highest to lowest were: Fe= 46.2%, Zn= 23.1%, Cu= 7.7%, B₆= 7.7%, B₁₂= 7.7%, and Fol= 3.8%. In 3.8% of the participants with anemia we did not find deficiencies related to iron homeostasis. There was a significantly higher number of participants with iron deficiency in the subpopulation with anemia compared to the total population (p= 0.04). There was no significant difference in the number of participants with zinc, copper, vitamin B₆, folate, or vitamin B₁₂ deficiencies between the total population and the subpopulation with anemia.
Prevalence of deficiencies in the subpopulation with three-day food records from highest to lowest were: Fe= 22.2%, Zn= 11.1%, Cu= 11.1%, B₆= 0%, Fol= 0%, and B₁₂= 0%. Prevalence of anemia in the subpopulation with three-day food records was 44.4%. In 55.5% of the population with dietary records we did not find deficiencies related to iron homeostasis.

Figure 2.1 shows that patients in the total population experienced isolated deficiencies (deficiencies occurring alone) and combined deficiencies (multiple deficiencies occurring simultaneously) in nutrients involved in iron homeostasis. In the total population, iron and zinc deficiency occurred in isolation as well as in combination with other micronutrient deficiencies. Iron deficiency occurred in isolation (prevalence 19%) and in combination with deficiencies in zinc and vitamin B₁₂ (prevalence 6%). Zinc deficiency occurred in isolation (prevalence 7%) or combined with deficiencies in other nutrients, including iron, copper, vitamin B₆, folate, and vitamin B₁₂ (prevalence 13%). Deficiencies in the other nutrients (copper, vitamin B₆, folate, and vitamin B₁₂) occurred in combination with other nutrient deficiencies only.

Figure 2.2 shows that patients in the population with anemia experienced isolated and combined deficiencies in nutrients implicated in iron homeostasis and anemia. There was a higher prevalence of deficiencies in nutrients other than iron, but that were still involved in iron homeostasis and metabolism, than iron deficiency. Participants with anemia presented with isolated iron deficiency (prevalence 35%), as well as iron deficiency combined with deficiencies in zinc and vitamin B₁₂ (prevalence 12%). Nutrient deficiencies other than iron deficiency
were present in combination only (prevalence 49%); nutrients included zinc, vitamin B₆, folate, vitamin B₁₂ and copper. In 4% of the population with anemia (one participant), we did not detect any deficiencies in nutrients related to iron nutriture.

Normal or deficient status of iron, zinc, copper, vitamin B₆, folate, or vitamin B₁₂ was not associated with the number of years since surgery. Concentration of iron, zinc, copper, vitamin B₆, folate, or vitamin B₁₂ was not correlated with the number of years since surgery.

2.5.3 Blood Cell Size in Participants with Anemia

Within the subpopulation with anemia, 15% of participants presented with microcytic anemia (MCV < 78 fL) and 85% presented with normocytic anemia (MCV 78-100 fL). Of the four participants with microcytic anemia, one was deficient in iron only, one was deficient in zinc only, one was deficient in iron and zinc only, and in one patient we did not assess micronutrient deficiencies.

2.5.4 Dietary Intake

The average intakes of macronutrients, as well as micronutrients that impact iron absorption and homeostasis are shown in Table 2.3. On average patients reported a daily intake of total energy= 1650.4 ± 605.1 kcal, total fat= 66.5 ± 10.2 g, total protein= 77.9 ± 9.8 g, animal protein= 57.8 ± 8.6 g, and total carbohydrate= 189.8 ± 28.9 g. Daily micronutrient intake averaged total iron= 41.8 ± 18.2 mg, heme iron= 0.85 ± 0.18 mg, non-heme iron= 40.9 ± 18.3 mg,
copper = 2.6 ± 0.9 mg, zinc = 19.7 ± 5.3 mg, calcium = 1407.8 ± 341.0 mg, vitamin C = 108.0 ± 30.6 mg, vitamin B₆ = 3.1 ± 0.8 mg, folate = 594.2 ± 169.1 mcg, and vitamin B₁₂ = 119.8 ± 112.5 mcg. This population, on average, exceeded the RDAs for all nutrients assessed, including vitamin C which enhances iron absorption, and calcium which inhibits iron absorption. However, we did not find significant associations or correlations between micronutrient intake and iron status.

We found associations between intake of macronutrients and iron status. Total energy intake (p= 0.009), as well as fat (p= 0.026), protein (p=0.013), and animal protein (p=0.023), was higher in subjects with normal compared to low ferritin status (Figure 2.3). We did not find significant associations between iron status (overall iron deficiency status, and status of iron deficiency measured by ferritin, TIBC, sTfR and sTfR:ferritin ratio) or anemia, and dietary intake of total iron, heme iron, or non-heme iron (data not shown). Dietary heme iron intake was correlated with years post-RYGB surgery (r=0.67, p<0.05; figure 2.4); for each increase in years post surgery, dietary iron intake increased by 0.67 ± 0.28 mg. There was no association between meat servings and iron status. There were no associations between intake of other micronutrients or macronutrients and time following surgery.

2.6 Discussion

In summary, our main findings were that following RYGB, more than half of the patient population presented with deficiencies related to iron nutriture,
including deficiencies in iron, zinc, copper, vitamin B₆, folate, and vitamin B₁₂; prevalence of deficiency was not related to the number of years since surgery. Of importance, simultaneous deficiencies were common. Notably, of the isolated deficiencies, iron was most prevalent. Similarly, patients with anemia had isolated iron deficiency as well as simultaneous deficiencies in iron, zinc, copper, vitamin B₆, folate, and vitamin B₁₂; deficiencies in nutrients other than iron were more common than isolated iron deficiency in the subpopulation with anemia. In addition, RYGB patients, on average, exceeded the RDAs for nutrients involved in iron homeostasis, as well as nutrients known to inhibit iron absorption; however, there was no association between the intake of micronutrients and iron status or anemia. RYGB patients with low iron stores (indicated by ferritin concentrations) consumed less total energy, fat, protein, and animal protein than patients with normal iron stores, suggesting that the intake of macronutrients had a greater impact on iron status than the intake of micronutrients. Finally, dietary heme iron intake increased with time following RYGB surgery, suggesting that gastric bypass patients consume more meat further out from surgery.

Consistent with recent literature, we have documented deficiencies in iron, zinc, copper, vitamin B₁₂, folate, and vitamin B₆ following RYGB. Compared to recent studies, most of which have followed RYGB surgery cohorts for up to two years post-surgery, our findings show the prevalence of deficiencies in nutrients implicated in iron homeostasis in RYGB patients who are, on average, five years post-surgery. Since we measured patients who were further out from surgery, our reported prevalence
of deficiency may be lower, compared to other studies, for nutrients such as folate= 15%, vitamin B₁₂= 50%, vitamin B₆= 18%, and copper= 10%. However, it is also difficult to compare prevalence between studies because of the variability in cutoffs and methods used in measuring micronutrients. Another theory is that the status of nutrients may increase with lessened food intolerance in the years following surgery; however, our findings suggest that micronutrient status is not associated with the time that has elapsed since surgery.

Other novel findings from the current study are the combinations in which these deficiencies are presented, both in the general RYGB population and in RYGB population with anemia. Iron deficiency and its clinical manifestation, anemia, can occur simultaneously with deficiencies in other nutrients, including zinc and B vitamins. Also, in addition to iron deficiency, patients with anemia presented with deficiencies in copper, zinc, vitamin B₆, folate and vitamin B₁₂. Therefore, iron deficiency may mask these other forms of malnutrition if it is the only nutrient that is measured. The occurrence of simultaneous deficiencies also makes it difficult to use MCV to differentiate between the types of anemia. Eighty-five percent of the population with anemia presented with normocytic anemia, 15% presented with microcytic anemia (generally attributed to deficiencies in iron, copper, vitamin B₆, and zinc), and no patients presented with macrocytic anemia (generally associated with megaloblastic anemias of folate and vitamin B₁₂). It is possible that most of our patients presented with normocytic anemia because having microcytic and macrocytic anemias simultaneously may blunt the phenotypic effects of either type of anemia and have an overall appearance of...
normal cell size. More specific tests are needed for accurate differentiation; examining megalocytes and segmented neutrophils on a peripheral blood smear may better detect megaloblastic anemias.

We found a significant positive correlation between dietary heme iron intake and the number of years post-surgery. This finding suggests that participants who were further out from surgery consumed more heme iron, which is found in meat products. Initially, this appeared to be inconsistent with findings of meat intolerance with little improvement up to seven years post-surgery\textsuperscript{100}; however, after removing time points greater than seven years post-surgery from our study population, heme iron intake was no longer associated with the number of years post-surgery. Therefore, heme iron intake may initially increase with the increase in time following surgery (up to seven years), but heme iron intake may ultimately plateau. Although heme iron is normally considered to be the more bioavailable form of iron, malabsorptive procedures delay interaction between pancreatic enzymes and biliary secretions and the food bolus until the common channel. This has a pronounced adverse effect on heme iron absorption, as biliopancreatic secretions are necessary for proteolytic digestion of myoglobin and hemoglobin, and ultimately the release of heme that is available for absorption.\textsuperscript{89,108} This phenomenon may account for why iron status did not improve with the increase in heme iron take, and why iron status did not improve with time following surgery.

Our patient population exceeded the RDAs for all nutrients assessed, including iron, copper, zinc, vitamin B\textsubscript{6}, vitamin B\textsubscript{12}, folate, calcium, and vitamin C.
However, the 2013 Update of the Clinical Practice Guidelines for the Perioperative Nutritional, Metabolic, and Nonsurgical Support of the Bariatric Surgery Patient recommends that RYGB patients take two adult multivitamin plus mineral supplements daily. The guidelines further specify minimum daily supplementation of 45-60 mg iron, 2 mg of copper, 400 mcg of folate, and 1000 mcg of vitamin B$_{12}$. There were no specific recommendations for vitamin B$_6$ and zinc. Our study population did not reach these recommended supplementation levels for iron or vitamin B$_{12}$. However, we found no association between the intake of micronutrients and iron status or anemia, which suggests that the intake of micronutrients may not be used to predict or treat iron deficiency or anemia.

A notable strength of this study was recruitment from the community, rather than from one bariatric center, which is typical in the literature. Recruiting participants from the community afforded us a representative sample of bariatric surgery patients who obtained their surgical procedures at various clinics, who had various lengths of elapsed time since surgery, and who had different dietary recommendations following surgery. This representative RYGB population sample enabled us to accurately measure the true prevalence of nutrient deficiencies and dietary intake. A limitation was that our study was cross-sectional. As a result, we cannot infer any incidence or cause-and-effect relationships; we can only present associations. Another limitation of our study was our sample size for the subpopulation with three-day food records; the low number of participants in this group may explain the lack of association between the intake of micronutrients from diet and supplements, and iron status or anemia.
Although iron deficiency is prevalent following RYGB, there are patients who remain normal in iron status. Therefore, our goal was to investigate other factors that may affect iron status following surgery, including the status and dietary intake of other nutrients implicated in iron homeostasis. In support of this goal, we found that there were deficiencies in micronutrients involved in iron absorption (copper), heme synthesis (vitamin B₆ and zinc) and blood cell differentiation (vitamin B₁₂ and folate). We found that combinations of deficiencies in these nutrients were more prevalent than isolated iron deficiency. Additionally, we found that dietary intake of energy, fat, total protein, and animal protein were lower in individuals with low iron stores compared to individuals with normal iron stores. Finally, we found that patients consumed more heme iron the further they were out from surgery. Comprehensive screening of micronutrient status following RYGB surgery is needed in order to uncover defects in iron nutrition following RYGB. Also, increasing consumption of foods with high content of calories, fat and protein may predict a more favorable iron status, but may have undesirable effects on other surgery outcomes such as weight regain. These findings may be used to guide further investigation and develop prophylactic measures.
Table 2.1 Population Characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total Population</th>
<th>Population with Anemia</th>
<th>Population with Dietary Records</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size</td>
<td>N=70</td>
<td>N=26</td>
<td>N=9</td>
</tr>
<tr>
<td>% Female</td>
<td>91</td>
<td>100</td>
<td>89</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td>79: 14: 1</td>
<td>75: 25: 0</td>
<td>100: 0: 0</td>
</tr>
<tr>
<td>(% White: Black: Other)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in Years (range)</td>
<td>49.4 ± 0.9 (30 to 65)</td>
<td>50.3 ± 1.2 (35 to 61)</td>
<td>48.0 ± 2.6 (30 to 58)</td>
</tr>
<tr>
<td>Years Post Surgery (range)</td>
<td>*4 ± 2 (0 to 33)</td>
<td>*4 ± 2 (0 to 12)</td>
<td>5.4 ± 1.7 (0 to 14)</td>
</tr>
<tr>
<td>BMI (kg/m²) (range)</td>
<td>*28.9 ± 2.7 (18.2 to 61.1)</td>
<td>*26.4 ± 2.5 (21.0 to 61.1)</td>
<td>*26.5 ± 2.7 (22.3 to 45.0)</td>
</tr>
<tr>
<td>Multivitamin Use</td>
<td>51: 10: 39</td>
<td>50: 12: 38</td>
<td>78: 22: 0</td>
</tr>
<tr>
<td>(% Yes: No: Missing)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1 shows the population characteristics for the total population of screened RYGB participants, the subpopulation with anemia, and the subpopulation with completed dietary records. Age in Years, Years Post Surgery, and BMI reported as mean ± S.E.M for normally distributed data and *median ± semi-interquartile range for skewed data.
Table 2.2 Prevalence of Deficiencies

<table>
<thead>
<tr>
<th>Type of Deficiency</th>
<th>Total Population (N=70) number: prevalence</th>
<th>Population with Anemia (N=26) number: prevalence</th>
<th>Population with Dietary Records (N=9) number: prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>17: 24.3%</td>
<td>12: 46.2%</td>
<td>2: 22.2%</td>
</tr>
<tr>
<td>Zinc deficiency</td>
<td>14: 20.0%</td>
<td>6: 23.1%</td>
<td>1: 11.1%</td>
</tr>
<tr>
<td>Copper deficiency</td>
<td>2: 2.9%</td>
<td>2: 7.7%</td>
<td>1: 11.1%</td>
</tr>
<tr>
<td>Vitamin B₆ deficiency</td>
<td>3: 4.3%</td>
<td>2: 7.7%</td>
<td>0</td>
</tr>
<tr>
<td>Folate deficiency</td>
<td>1: 1.4%</td>
<td>1: 3.8%</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin B₁₂ deficiency</td>
<td>2: 2.9%</td>
<td>2: 7.7%</td>
<td>0</td>
</tr>
<tr>
<td>No deficiency or deficiency in other nutrients</td>
<td>31: 44.3%</td>
<td>1: 3.8%</td>
<td>5: 55.5%</td>
</tr>
</tbody>
</table>

Table 2.2 shows the types and prevalence of nutrient deficiencies related to iron homeostasis in the total screened RYGB population, the subpopulation with anemia, and the subpopulation with completed dietary records.
Figure 2.1. Combined deficiencies in nutrients related to iron homeostasis in the total population (N=70). Abbreviations: Fe= iron, Cu= copper, Zn= zinc, B6= vitamin B$_6$, Fol= folate, B12= vitamin B$_{12}$. 
Figure 2.2. Combined deficiencies in nutrients related to iron homeostasis in the subpopulation with anemia (N=26). Abbreviations: Fe= iron, Cu= copper, Zn= zinc, B6= vitamin B6, Fol= folate, B12= vitamin B12.
### Table 2.3 Mean daily dietary intake of post-RYGB patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± S.E.M.</th>
<th>% RDA for average participant: (Female, Age 31-50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Energy (kcal)</td>
<td>1650.4 ± 605.1</td>
<td>-</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>66.5 ± 10.2</td>
<td>-</td>
</tr>
<tr>
<td>Total Protein (g)</td>
<td>77.9 ± 9.8</td>
<td>-</td>
</tr>
<tr>
<td>Animal Protein (g)</td>
<td>57.8 ± 8.6</td>
<td>-</td>
</tr>
<tr>
<td>Total Carbohydrate (g)</td>
<td>189.8 ± 28.9</td>
<td>-</td>
</tr>
<tr>
<td>Total Iron (mg)</td>
<td>41.8 ± 18.2</td>
<td>232.2%</td>
</tr>
<tr>
<td>Heme Iron (mg)</td>
<td>0.85 ± 0.18</td>
<td>-</td>
</tr>
<tr>
<td>Non-heme Iron (mg)</td>
<td>40.9 ± 18.3</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>3.1 ± 0.8</td>
<td>238.5%</td>
</tr>
<tr>
<td>Folate (mcg)</td>
<td>594.2 ± 169.1</td>
<td>148.6%</td>
</tr>
<tr>
<td>Vitamin B₁₂ (mcg)</td>
<td>119.8 ± 112.5</td>
<td>4991.6%</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>108.0 ± 30.6</td>
<td>144.0%</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>2.6 ± 0.9</td>
<td>288.9%</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>19.7 ± 5.3</td>
<td>246.2%</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1407.8 ± 341.0</td>
<td>140.8%</td>
</tr>
</tbody>
</table>

Table 2.3. Dietary intake was analyzed using NDSR software. Average ± S.E.M. was determined using STATISTCA software. The %RDA was calculated using the RDA for the average study participant, who was female and age 31-50.
Figure 2.3. Associations between ferritin status as mean total energy, total fat, total protein, and animal protein in nine RYGB patients. Normal ferritin status (dark grey bars) was defined as ferritin ≥ 20 mcg/L and deficient ferritin status (light grey bars) was defined as ferritin < 20 mcg/L.
Figure 2.4. Correlation ($r=0.67$, $p<0.05$) between dietary heme iron intake and years post-RYGB surgery in nine participants. Heme iron was calculated by multiplying the iron content in meat products by 0.4. The dashed lines represent the 95% confidence interval for the Pearson’s correlation.
CHAPTER 3. CONCLUSIONS

3.1 Conclusions

Fifty-six percent of the total RYGB study population presented with micronutrient deficiencies related to iron nutriture; this includes deficiencies in iron, copper, zinc, vitamin B₆, folate, and vitamin B₁₂. This suggests that, more often than not, patients will present with one or more deficiencies in nutrients that affect iron status post-operatively. In addition, there is a high prevalence of anemia (37%) in the RYGB population, which would be expected since deficiency in iron and iron-related nutrients are so common. Of importance, RYGB patients more commonly present with multiple deficiencies simultaneously in nutrients related to iron homeostasis than with iron deficiency alone. Therefore, it is vital that health providers for RYGB patients routinely perform comprehensive assessments of micronutrient status, which includes iron as well as zinc, copper, and the B vitamins, in order to detect and effectively treat iron deficiency and anemia following surgery.

Dietary intake of iron (heme or non-heme) or of other micronutrients did not appear to affect iron status or anemia. Therefore, it may not be effective to make dietary recommendations to increase intake of iron and related nutrients through diet and use of supplements in order to improve iron status or anemia;
this must be confirmed in future studies. However, patients with normal ferritin concentrations had a higher consumption of total energy, fat, protein, and animal protein than patients with low ferritin concentrations. Although a more favorable iron status is associated with higher fat consumption, recommending higher fat consumption may have undesirable effects; increasing dietary fat consumption may contribute to weight regain in the bariatric surgery population, as dietary fat induces overconsumption and weight gain in obese and post-obese subjects. However, it may be worthwhile to recommend a high-protein diet in RYGB patients to improve iron status. Since we found that heme iron intake increases with time following surgery, it appears plausible to increase animal protein (found in meat, which is the primary source of heme iron) in the years following surgery. However, if patients are intolerant to animal protein in the immediate period following surgery, other forms of protein supplementation may be needed in order to improve iron status in these patients.

Our findings supported our hypothesis that iron deficiency occurs in combination with deficiencies of other nutrients that effect iron status. These nutrients include vitamin B$_{12}$ and zinc. However, RYGB patients also presented with iron deficiency alone, zinc deficiency alone, or multiple deficiencies in nutrients excluding iron. Our findings did not support our hypothesis that dietary intake of iron or other nutrients involved in iron homeostasis is an important factor in determining iron status. Instead, our preliminary data suggests that macronutrient, rather than micronutrient, intake impacts iron status. Our findings did support our hypothesis that anemia is associated with iron deficiency as well
as deficiencies in other nutrients following RYGB. RYGB patients with anemia also experienced multiple deficiencies simultaneously more often than they experienced iron deficiency alone.

Implications of these findings are that clinicians who evaluate post-RYGB patients, especially patients who present with known symptoms or manifestations of iron deficiency, should include copper, zinc, vitamin B₆, folate, and vitamin B₁₂ in their assessment. Although the latest Clinical Practice Guidelines for the Perioperative Nutritional, Metabolic, and Nonsurgical Support of the Bariatric Patient recommends postoperative evaluation of iron, zinc, and vitamin B₁₂, there are no recommendations for assessment of copper, folate, or vitamin B₆. For post-bariatric patients with anemia, these guidelines suggest evaluating vitamin B₁₂, folate, copper, and zinc only if routine screening for iron deficiency is negative;¹²⁸ this does not take into account the occurrence of multiple deficiencies simultaneously, which was shown by this study to be more prevalent than the occurrence of any deficiency in isolation. Even if a patient tests positive for iron deficiency, this does not rule out the possibility of simultaneous deficiencies in other nutrients. Therefore, failing to perform comprehensive assessment of all nutrients and/or treating some deficiencies and not others will not effectively treat these patients. Routine, comprehensive screening is important for prevention, accurate diagnosis, and effective treatment of micronutrient deficiencies following RYGB.
3.2 Future Directions

In the future, it would be beneficial to increase the number of participants with dietary records. This will allow us to have more definitive information regarding associations between dietary intake and iron status. We would also like to develop the appropriate micronutrient formula to effectively treat iron deficiency through supplementation. Our preliminary findings have suggested that RYGB patients exceed the RDA for intake of iron and related nutrients; however, RYGB patients are at higher risk for micronutrient deficiencies than the normal population. In support of this, the guidelines for bariatric surgery patients recommend that RYGB patients take two multivitamin plus mineral supplements daily, although they do not specify recommended intake of all nutrients related to iron nutriture. Thus, future studies are needed to determine effective doses of iron and other nutrients involved in iron homeostasis. Also, it would be useful to assess dietary intake of phytates and polyphenols that are known to inhibit iron absorption, in order to more thoroughly investigate the impact of food constituents on iron status.

Additionally, we are in the process of developing an in-house method for simultaneously quantifying water-soluble vitamins from plasma samples using mass spectrometry. This is a potential cost effective method that may be used to perform comprehensive analysis of nutrients involved in iron homeostasis, such as the B vitamins. We hope that lowering the cost of micronutrient assessment will facilitate the comprehensive screening that we propose will benefit the RYGB population.
Finally, recent evidence suggests that polymorphisms in genes that regulate iron absorption and mobilization promote iron deficiency. Single nucleotide polymorphisms (SNP)s in the transferrin gene, $TF$, type-2 transferrin receptor gene, $TFR2$, and the $TMPRSS6$ gene have been documented in humans and are associated with reduced iron status; thus genetics may also contribute to reduced iron status following RYGB. In the future, we would like to determine if SNPs are associated with measures of iron status in a cross-sectional study of RYGB patients.

It is estimated that, since 1990, 1.4 million persons have undergone bariatric surgery in the United States alone. Therefore, it is imperative that we continue to perform the investigations needed in order to optimize post-surgery health.
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