FOLATE AND IRON STATUS IN WOMEN OF CHILDBEARING AGE AFTER BARIATRIC SURGERY

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ABSTRACT

Women of childbearing age constitute a large proportion of bariatric surgery patients. Bariatric surgery is associated with increased risk of nutritional deficiencies. Adequate folate and iron status is important during pregnancy to ensure healthy infant outcomes. The primary purpose of this research was to determine the association between surgical procedure, Roux-en-Y gastric bypass (RYGB) vs. laparoscopic adjustable gastric banding (LAGB), and pre- to one year post-surgery change in serum folate and serum ferritin (an indicator of iron status) among women of childbearing age and to determine the prevalence of postoperative folate and iron deficiencies. There were 413 subjects (272 RYGB, 141 LABG) for the folate analyses and 426 subjects (280 RYGB, 146 LAGB) for the ferritin analyses. The subjects were a subset of participants from the Longitudinal Assessment of Bariatric Surgery study.

Type of surgery was not significantly associated with change in serum folate. Among women who underwent RYGB, daily/weekly multivitamin use at one-year follow-up and daily/weekly multivitamin use at both baseline and one-year follow-up were confounders of the relationship between RYGB and change in serum folate. The prevalence of folate deficiency one year after surgery was low, 0.7% after LAGB and 1.1% after RYGB. For change in serum ferritin, there were significant interactions between type of surgery and percent weight change

and baseline serum ferritin such that the associations of percent weight change and baseline ferritin were more pronounced among women who had undergone RYGB compared to LAGB. For women who underwent RYGB, greater percent weight change from baseline was associated with greater change in serum ferritin ($p<.0001$). The prevalence of iron deficiency was greater ($p=.04$) among women who underwent RYGB than LAGB (21.8% vs. 13.7%).

This research is relevant to public health because it provides evidence that RYGB presents a potential risk to perinatal health due to iron deficiency one year after surgery for women who become pregnant. The folate results should be interpreted with caution due to the use of serum folate, an indicator of short-term folate status and intake. Additional research is recommended using red blood cell folate concentration to determine folate status.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	analysis of variance test
AOR	adjusted odds ratio
AUDIT	Alcohol Use Disorders Identification Test
BMI	body mass index
BPD	biliopancreatic diversion
CI	confidence interval
DCC	Data Coordinating Center
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
GED	General Equivalency Degree or General Education Diploma
HCl	hydrochloric acid
HR	hazard ratio
LABS	Longitudinal Assessment of Bariatric Surgery study
LAGB	laparoscopic adjustable gastric banding
LSG	laparoscopic sleeve gastrectomy
MVI	multivitamin
NHANES	National Health and Nutrition Examination Survey
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases

Post-op	postoperative
Pre-op	preoperative
RBC	red blood cell
RDA	Recommended Dietary Allowance
RYGB	Roux-en–Y gastric bypass
SG	sleeve gastrectomy
TIBC	total iron-binding capacity
U.S.	United States of America

1.0 INTRODUCTION

1.1 BACKGROUND

An estimated 196,000 bariatric surgical procedures are performed each year in the United States.[1] Prospective studies in the U.S. have reported that approximately 80% of bariatric surgery patients are women.[2,3] Of these women, approximately 50% are in the childbearing age range of 18-45 years.[4,5] Prior to bariatric surgery, 30% of women of childbearing age reported future pregnancy was important to them and 33% of these women planned to become pregnant within two years of surgery.[6] In a large prospective cohort study, 45% of women reported unprotected intercourse during the first year after bariatric surgery.[7]

Having good nutritional status prior to pregnancy is important for healthy infant outcomes.[8] Two critical nutrients during the periconceptional period are folate and iron.[8] One of the recognized complications of bariatric surgery is an increased risk for micronutrient deficiencies.[9,10] The American Association of Clinical Endocrinologists, the Obesity Society, and the American Society for Metabolic and Bariatric Surgery recommend women avoid becoming pregnant for 12-18 months following bariatric surgery.[11] However, some researchers recommend that a longer period is needed between surgery and pregnancy to decrease the risk of negative infant outcomes.[12]

1.1.1 Overview of bariatric surgical procedures

Bariatric surgical procedures produce weight loss through a number of mechanisms, including reducing food intake related to altered levels of gut hormones and gastric volume restriction, changing food preferences, and intestinal malabsorption of macronutrients.[13,14] The most common procedures currently utilized in the U.S. are the sleeve gastrectomy, estimated to account for 53.8% of procedures performed, Roux-en-Y gastric bypass, estimated to account for 23.1% of procedures performed, and laparoscopic adjustable gastric banding, approximately 5.7% of procedures performed.[1]

In the sleeve gastrectomy (SG) (Figure 1[15]), approximately 80% of the stomach is surgically removed, leaving a narrow gastric tube which includes the pyloric sphincter.[16] This surgery promotes weight loss by increasing satiety due to the reduced size of the stomach pouch and decreased production of ghrelin, a gastrointestinal hormone that stimulates hunger, resulting in restricted food intake.[16-18]

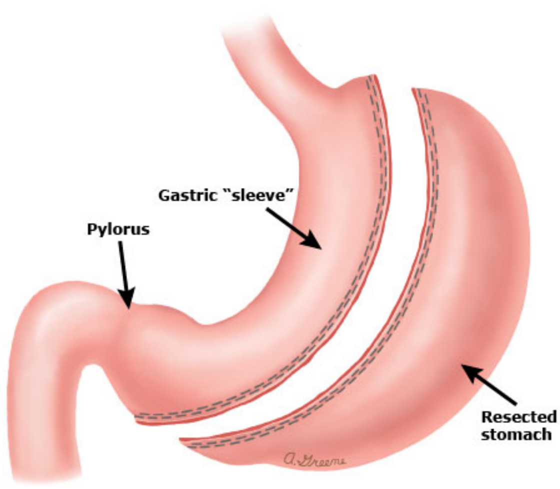


Figure 1: Sleeve gastrectomy

Laparoscopic adjustable gastric banding (LAGB) (Figure 2[19]) is a reversible procedure involving the placement of a hollow band around the upper portion of the stomach to create a small stomach pouch.[16] The size of the pouch can be adjusted by increasing or decreasing the amount of saline injected into the band through a subcutaneous port.[16] Weight loss results from decreased food intake related to increased satiety caused by the small size of the stomach pouch.[16]

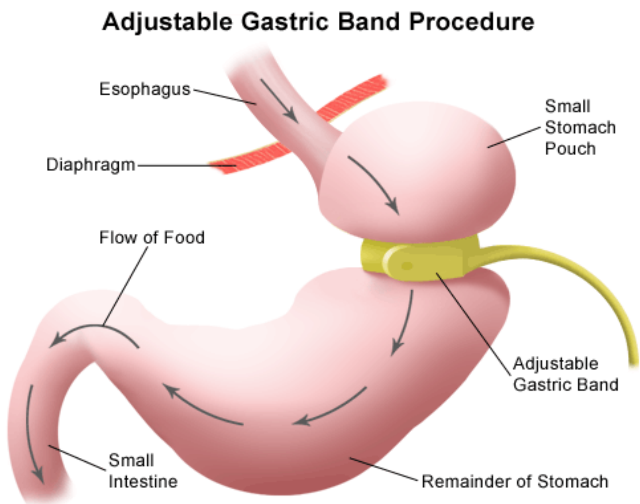


Figure 2: Laparoscopic adjustable gastric banding

For the Roux-en–Y gastric bypass (RYGB) (Figure 3[20]), the first component of the surgery is to create a small stomach pouch, with a volume of approximately 30 milliliters, using staples.[16,21] The second component involves intestinal surgery to reroute the passage of food from the gastric pouch directly to a segment of the jejunum bypassing the distal stomach, duodenum, and proximal jejunum.[16] The altered secretion of gastrointestinal hormones and smaller stomach pouch result in increased satiety and limit the amount of food that can be consumed at one time.[16,22] Weight loss is also achieved by altering the flow of nutrients through the proximal intestine, which decreases mixing time of nutrients with gastric acid, bile, and pancreatic enzymes, leading to the malabsorption of some macronutrients and thus, calories.[22] A large prospective study comparing weight change for patients who underwent RYGB versus LAGB at three years following surgery reported that RYGB participants lost 31.5% of baseline weight compared to only 15.9% for LAGB participants (note: weight change data for participants who had undergone SG were not provided).[4]

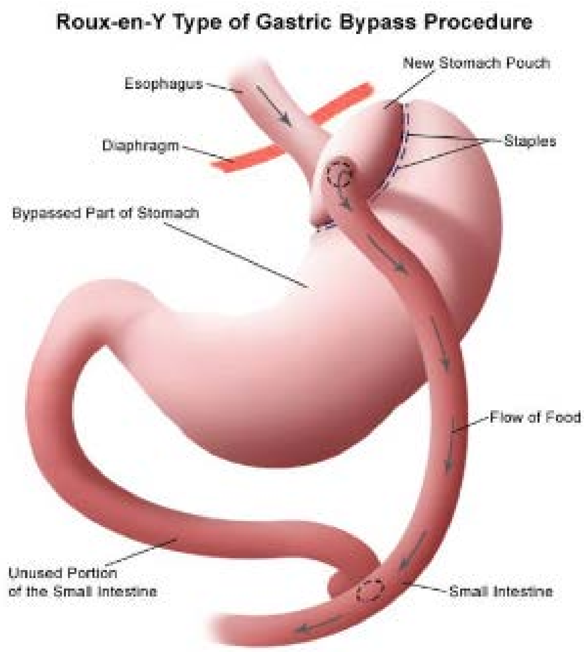


Figure 3: Roux-en-Y gastric bypass

1.1.2 Bariatric surgery and micronutrient deficiencies

Micronutrient deficiencies are well-known complications of bariatric surgery.[9,10,23-26] Patients who have undergone bariatric surgery are at risk for deficiencies of vitamin B_{12}, thiamin, folate, vitamin C, vitamins A, D, and K, calcium, iron, zinc, and copper.[9,10] Some studies report on serum ferritin which reflects iron stores in the body and is used as an indicator of early iron deficiency.[27] The mechanism of action of deficiency varies for each specific micronutrient but includes: decreased oral intake due to smaller stomach capacity, increased satiety, food intolerances (e.g. meat), and dietary restrictions, frequent vomiting, noncompliance with multivitamin (MVI) and minerals supplementation recommendations, and altered nutrient absorption due to intestinal bypass or decreased secretion of hydrochloric acid (HCl) or intrinsic factor, needed for absorption of iron and vitamin B_{12}, respectively.[9,10,22] The risk of micronutrient deficiency varies based on the type of surgery, with the surgeries that are the most successful in producing weight loss being more likely to result in micronutrient deficiencies.[24] Micronutrient deficiencies are more common after biliopancreatic diversion (BPD) and RYGB due to the malabsorptive component of the surgeries.[23,25,26]

Due to the risk of deficiencies, the American Society for Metabolic and Bariatric Surgery has provided recommendations for postoperative micronutrient supplementation.[23,28] For individuals who have undergone LAGB, a MVI with minerals supplement that provides 100% of the daily value for at least two-thirds of the nutrients including folate, iron, zinc, and selenium is recommended.[23] After RYGB, it is recommended to take a MVI with minerals supplement that provides 200% of the daily value for at least two-thirds of the nutrients.[23] This is often achieved by taking two MVI with minerals supplements per day. It is recommended that individuals who have undergone bariatric surgery receive 400-800 µg of folate daily from a MVI with minerals; while, women of childbearing age should take 800-1000 µg of folate daily.[28] Women who are menstruating or have undergone RYGB are recommended to take at least 45-60 mg of elemental iron daily as a combination of iron provided by the MVI with minerals supplement and an individual supplement; otherwise, 18 mg of iron from a MVI with minerals supplement is recommended.[28] Individuals who have undergone LAGB or RYGB should take at least 12 mg of thiamin daily, 350-500 µg of sublingual or liquid vitamin B_{12} or 1000 µg of intramuscular vitamin B_{12} monthly, 1200-1500 mg of calcium, and vitamin D supplementation based on serum levels.[28]

1.1.2.1 Micronutrient deficiencies in adults with obesity

When examining the prevalence of micronutrient deficiencies resulting from bariatric surgery, it is important to acknowledge candidates for bariatric surgery may have preexisting deficiencies. While it may seem counterintuitive that individuals with obesity would have micronutrient deficiencies, studies have proven otherwise.[29-35] Individuals with obesity may consume foods high in calories, sugar, and fat but low in micronutrient density.[36] In addition, the bioavailability of certain micronutrients may be decreased due to altered absorption, distribution, metabolism, or excretion processes in individuals with obesity.[37] Table 1 provides a summary of prevalence data on micronutrient deficiencies for adults with obesity (note: only data for women is included in the table).

Table 1: Summary of findings on prevalence of micronutrient deficiency specifically for women with obesity

Author, year (location)	Female subjects only	Micronutrients assessed (serum level used to define deficiency)	Prevalence of deficiency
Kimmons, 2006[30] (United States)	1320 premenopausal women (mean age 34.5 years)	folate (serum folate <3 ng/mL, RBC folate <109 ng/mL) vitamin A (≤30 µg/dL) total carotenoids (NR) vitamin B_{12} (≤299 pg/mL) vitamin C (<0.4 mg/dL) vitamin D (≤15 ng/mL) vitamin E (NR) selenium (<100 µg/L)	32%, 18% 2% 40% 17% 35% 19% 26% 6%
Nicoletti, 2013[33] (Brazil)	65 women (mean age specifically for women NR)	folate (<3 ng/mL) iron (ferritin <6 ng/mL, serum iron <40 µg/dL) vitamin A (<20 µg/dL) beta-carotene (<40 µg/100 dL) vitamin B_{12} (<174 pg/mL) vitamin C (<0.3 mg/dL) copper (<70 µg/dL) magnesium (<1.4 mEq/L) zinc (<50 µg/dL)	0% 0%, 11% 11% 0% 2% 12% 0% 22% 0%
Flancbaum, 2006[29] (United States)	320 women (mean age NR)	iron (ferritin NR, serum iron NR) thiamin (NR) vitamin B_{12} (NR) vitamin D (NR) calcium (NR)	10%, 42% 29% 0% 68% 4%
Ernst, 2009[35] (Switzerland)	165 women, (mean age 40 years) Subgroup: 66 women (mean age 40.5 years)	folate (<4.5 nmol/L equivalent to <1.99 ng/mL) iron (serum ferritin <18 pmol/L equivalent to <8 ng/mL) vitamin B_{12} (<133 pmol/L) vitamin D (<76 nmol/L) magnesium (<0.7 mmol/L) phosphate (<0.8 mmol/L) zinc (<11 µmol/L) vitamin A (<0.7 µmol/L) thiamin (<933 nmol/L) niacin (<65 µmol/L) vitamin B_6 (<33.2 nmol/L) vitamin E (<12 µmol/L) copper (<13 µmol/L) selenium (<0.9 µmol/L)	3% 9% 19% 90% 5% 7% 26% 0% 0% 6% 3% 3% 0% 30%

Abbreviations: RBC: red blood cell; NR: not reported

Table 1 (continued): Summary of findings on prevalence of micronutrient deficiency specifically for women with obesity

Author, year (location)	Female subjects only	Micronutrients assessed (serum level used to define deficiency)	Prevalence of Deficiency
Schweiger, 2010[32] (Israel)	83 women (mean age specifically for women NR)	folate (<5.6 ng/mL) iron (ferritin <20 ng/mL, serum iron <60 µg/dL)	26% 32%, 41%
Sanchez, 2016[34] (Chile)	103 women (mean age 36 years)	iron (ferritin<12 µg/L equivalent to <12 ng/mL, serum iron <50 µg/dL, transferrin saturation <16%) calcium (<8.5 mg/dL) copper (<70 µg/dL) phosphorus (<2.5 mg/dL) zinc (<60 µg/dL)	9% 13%, 15% 13% 0% 2% 3%
	Subgroup: 66 subjects (mean age NR)	folate (<1.5 ng/mL) vitamin B_{12} (<200 pg/mL) vitamin D (<20 ng/mL)	0% 11% 46%

Abbreviations: NR: not reported

Using National Health and Nutrition Examination Survey (NHANES) data from 1988-1994, Kimmons et al.[30] determined the prevalence of low micronutrient serum levels by body mass index (BMI) category (normal weight, overweight, and obese), age group, and gender. The prevalence of deficiency for premenopausal women with obesity (n=1320) are presented in Table 1. In comparison, the prevalence of deficiency for premenopausal women having a normal weight based on BMI (n=1980) was 16% in folate based on serum folate and 18% based on RBC folate, 17% in vitamin E, 11% in total carotenoids, 20% in vitamin C, 3% in selenium, 1% in vitamin A, 8% in vitamin D, and 18% in vitamin B_{12}. For premenopausal women, increasing BMI category was associated with lower serum levels of folate, vitamin E, total carotenoids, vitamin C, selenium, and vitamin D ($p<.05$, linear trend). Of note, the NHANES data for this study were obtained prior to the 1998 initiation of the Food and Drug Administration's mandatory folate fortification program for enriched grains and cereals.

The studies listed in Table 1 provide evidence that deficiency of micronutrients, including folate and iron, is not uncommon among women with obesity. The prevalence of micronutrient deficiencies in females with obesity ranged from 0% - 32% [30,32-35] for folate, 0% - 42% [29,32-35] for iron, 35-90% for Vitamin D[29,30,34,35], 0-26% for zinc[33-35], and 2-19% for vitamin B_{12}[29,30,33-35]. The subjects in the majority of these studies were candidates for bariatric surgery with prevalence of deficiencies determined prior to bariatric surgery. The biomarker used to identify deficiency varied for folate (serum folate or RBC folate) and iron (serum ferritin, serum iron, or transferrin saturation). In addition, the laboratory cutoffs to define deficiencies varied among studies, which leads to differences in the prevalence of deficiency.

It is plausible that some of those with deficiency after bariatric surgery may have had preoperative deficiency or a preoperative deficiency that was worsened by the decreased food intake and malabsorption caused by bariatric surgery. In addition, treating deficiencies with micronutrient supplementation may affect the postoperative serum levels. Therefore, determining folate and iron status prior to surgery is important context when reporting the prevalence of postoperative deficiencies.

1.1.3 Concerns with pregnancy after bariatric surgery

Due to rapid weight loss following bariatric surgery and the potential for maternal nutrient deficiencies, there is concern for the safety of the fetus among women who become pregnant after bariatric surgery.[38] Results of studies have been mixed, with earlier case reports (1986-1996) showing complications such as neural tube defects, anemia, and intrauterine growth retardation; while, later larger studies (2004-2010) have not reported an increased risk of perinatal complications following bariatric surgery.[38,39] Kjaer and Nilas[39] postulated that adverse obstetric outcomes may be due to obesity rather than the surgery itself. A recent matched retrospective cohort study by Hammeken et al.[40] reported a higher risk of giving birth to small-for-gestational-age infants and maternal anemia among women who had undergone RYGB compared to controls who did not have a history of RYGB. In another retrospective cohort study, Parent et al.[12] reported a greater risk of prematurity, neonatal intensive care unit admission, and small-for-gestational-age status for infants of mothers who had bariatric surgery compared to infants of mothers who did not have prior bariatric surgery. In addition, the risk of infant prematurity, neonatal intensive care admission, and small-for-gestational-age newborns was higher for women who gave birth less than two years after surgery compared to women who gave birth greater than four years after bariatric surgery.[12]

Due to the potential risk to fetal health, the American Association of Clinical Endocrinologists, the Obesity Society, and the American Society for Metabolic and Bariatric Surgery recommend that women avoid becoming pregnant for 12-18 months following bariatric surgery.[11] Based on the results of their study, Parent et al.[12] suggested that the recommended minimum safe interval from bariatric surgery to infant birth should be increased to three years. However, in a study by Menke et al.[7], 3.5% of women reported trying to conceive during the first year following bariatric surgery. The *Committee on Obstetric Practice of the American College of Obstetricians and Gynecologists* recommends women who have undergone bariatric surgery and are pregnant, or are planning to become pregnant, should be evaluated for potential deficiencies in iron, folate, vitamin B_{12}, vitamin D, and calcium.[41]

1.1.4 Importance of periconceptional folate status

Folate, a water-soluble B vitamin, functions in the body as a cosubstrate in many reactions involving amino acids and nucleotides, such as in the synthesis and repair of DNA.[42] Folate is required for the formation and maturation of erythrocytes and white blood cells, the conversion of homocysteine to methionine, normal cell division, and embryo and fetal development.[42] It has long been recognized that inadequate maternal folate status prior to, and early in, pregnancy is associated with the development of neural tube defects such as spina bifida.[43] Inadequate maternal folate status during pregnancy is also associated with higher risk for fetal growth retardation, low birth weight, and preterm delivery. [44]

1.1.4.1 Folate deficiency

Folate deficiency can be caused by a poor quality diet, alcoholism, drug-nutrient interactions, and malabsorptive disorders such as celiac disease and Crohn's disease.[45,46] Individuals who suffer from alcohol abuse commonly experience negative folate balance or folate deficiency.[47] Alcohol has numerous negative effects on folate status such as decreased intestinal absorption, inadequate utilization in the body, and greater urinary excretion.[47,48] Individuals with alcoholism often have decreased dietary intake of folate due to poor quality diets.[47,48] Despite the Food and Drug Administration's mandatory folate fortification program for enriched grains and cereals which began in 1998, there are still some populations in the U.S. at risk for inadequate intake of folate such as non-Hispanic black women.[49]

Food sources of folate and folic acid include green vegetables such as spinach, asparagus, and Brussels sprouts, fresh fruit and fruit juice, and fortified cereals, breads, pasta, and rice.[50] Folate is found naturally in foods in polyglutamate forms and also as folic acid, the monoglutamate form used in fortification.[49,51] The polyglutamate forms must first be hydrolyzed by enzymes in the proximal small intestine to the monoglutamate form prior to absorption. Folic acid does not require digestion and is readily absorbed.[51] In the body, folate is found in a number of active, interconvertible forms.[51] Examples of the active forms of folate and metabolic functions of each are included in Table 2.

Table 2: Active forms of folate and metabolic functions

Table 9.3 Forms of Folate and Their Metabolic Roles in the Body

Folate Form	Roles
10-formyl THF	**Folate transfers formate as 10-formyl THF for purine synthesis:** 5-phosphoribosylglycinamide ribonucleotide (GAR) conversion to 5-phosphoribosyl formylglycinamide (FGAR) by glycinamide ribonucleotide transformylase and 5-phosphoribosyl 5-amino 4-imidazole carboxyamide ribonucleotide (AICAR) conversion to 5-phosphoribosyl 5-formamido 4-imidazole carboxamide ribonucleotide (FAICAR) by aminoimidazolecarboxamide ribonucleotide transformylase
5, 10-methylene THF	**Folate transfers formaldehyde as 5,10-methylene for pyrimidine synthesis:** Deoxyuridine monophosphate (dUMP) conversion to deoxythymidine monophosphate (dTMP) by thymidylate synthetase **Folate receives formaldehyde for serine degradation/glycine synthesis:** Serine conversion to glycine by serine hydroxymethyltransferase **Folate receives formaldehyde for glycine degradation:** Glycine conversion to carbon dioxide and ammonium by the glycine cleavage system **Folate receives formaldehyde for glycine synthesis:** Dimethylglycine and its catabolic product sarcosine are degraded to glycine by dimethylglycine dehydrogenase and sarcosine dehydrogenase, respectively
5-formimino THF	**Folate receives a formimino group in histidine degradation:** Formiminoglutamate (FIGLU) conversion to glutamate by formiminotransferase
5-methyl THF	**Folate provides a methyl group for methionine synthesis:** Homocysteine conversion to methionine by methionine synthase

The primary methods for determining an individual's folate status are serum folate or RBC folate concentration.[52] Characteristics of these tests are presented in Table 3. Serum folate level is an indicator of short-term folate status, while RBC folate concentration reflects more long-term folate status.[52,53] The decision to use serum folate rather than RBC folate for the study reported here was because RBC folate concentration requires whole blood samples containing red blood cells, which were not available from LABS-2 participants. Other practical considerations are that the RBC assay is technically more difficult to perform and is more expensive than the serum folate assay.[54] Serum folate is also a better measure for individuals who have vitamin B_{12} deficiency.[54]

Table 3: Characteristics of laboratory tests used to diagnose folate deficiency

Test	Description	Criteria for diagnosing folate deficiency	Comments
Serum folate	Measures the amount of folate in the blood.[55]	<3 ng/mL, females and males[56,57]	Serum levels indicate short-term folate status and recent dietary intake.[51,55] Elevated serum folate may be caused by pernicious anemia.[55] Serum concentrations decrease during pregnancy.[58]
RBC folate	Measures the concentration of folate in red blood cells and is an indicator of tissue folate.[51,55]	<151 ng/mL, females and males[58]	RBC folate is an indicator of longer-term folate status and decreases after approximately 3-4 months of inadequate folate intake.[51] Vitamin B_{12} deficiency can cause falsely low RBC folate concentrations.[54,59] Serum concentrations decrease during pregnancy.[58]

Abbreviation: RBC: red blood cell

Based on 2003-2006 NHANES data, 23% of non-Hispanic black women in the U.S. did not meet the Dietary Reference Intake (DRI) estimated average requirement (EAR) for folate (including dietary supplement intake) compared to 13% of non-Hispanic white women ($p \leq .003$, Bonferroni-adjusted).[60] Analysis by age group found 17% of 19-30 year-old women and 15% of 31-50 year-old women in the U.S. had inadequate folate intake even with the use of dietary supplements.[60] The prevalence of folate deficiency, based on 1999-2010 NHANES data, is estimated to be 0.9% among childbearing-age women in the U.S.[61]

1.1.4.2 Bariatric surgery and folate deficiency

Folate deficiency has been identified in patients after bariatric surgery.[10,62-64] Folate deficiency can be caused by decreased intake of dietary sources and noncompliance with MVI supplementation.[23,65,66] Conversion of folate from the 5-methyl tetrahydrofolate form to the tetrahydrofolate form requires vitamin B_{12} to accept the methyl group (Figure 4).[51] Deficiency of vitamin B_{12} results in the "methyl-folate trap" and decreased synthesis of tetrahydrofolate.[51] Tetrahydrofolate is the form of folate needed for DNA synthesis.[51] The role of vitamin B_{12} deficiency is an important consideration since it is a common deficiency after bariatric surgery, with a reported prevalence of 62% at 5 years or more after RYGB.[67]

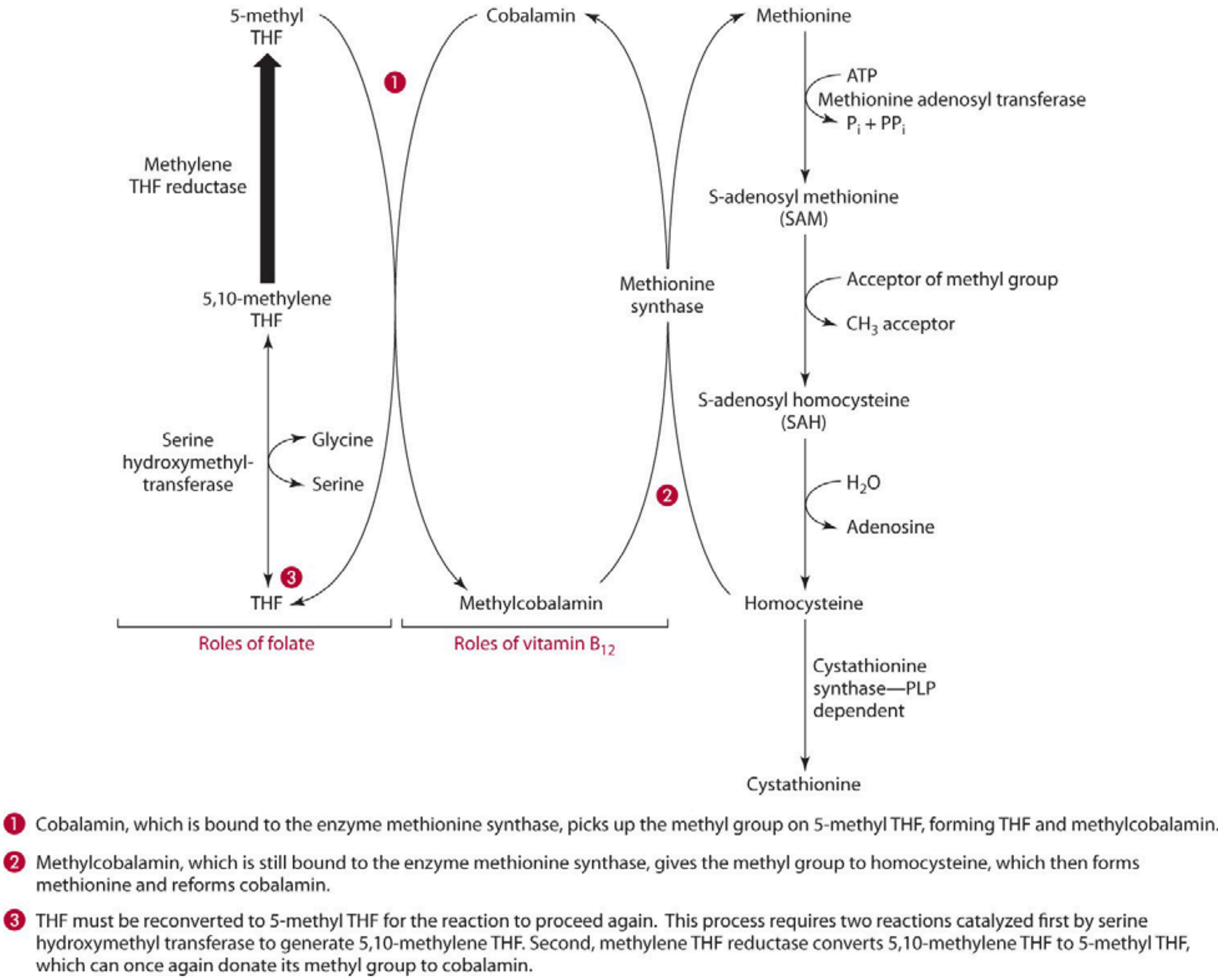


Figure 4: Role of vitamin B12 in folate metabolism
From Gropper/Smith. *Advanced Nutrition and Human Metabolism*, 6E. © 2013 Brooks/Cole, a part of Cengage, Inc. Reproduced by permission. www.cengage.com/permissions

1.1.5 Importance of periconceptional iron status

Iron is a trace element required for many essential body processes. It is required for the formation of hemoglobin in red blood cells and myoglobin in muscle, both of which are needed for oxygen transport.[68] It is an active component of many enzymes and cytochromes and is involved in oxidation and reduction reactions.[68] Deficiency of iron results in iron deficiency anemia, growth abnormalities, decreased muscle function and exercise tolerance, fatigue, epithelial disorders, and impaired cognitive development in children.[69] Periconceptional iron deficiency increases the risk of iron deficiency anemia in the mother during pregnancy and low infant iron stores at birth.[70] Maternal anemia early in pregnancy has been associated with greater risk of preterm birth[71-73] and low birth weight[71]. Perinatal iron deficiency interferes with the neurodevelopment of the fetus, resulting in difficulties with learning, language skills, emotional development, and motor skills.[74,75]

1.1.5.1 Iron deficiency

Iron deficiency can be caused by inadequate intake of dietary sources, acute or chronic blood loss, inadequate absorption due to malabsorptive disorders or achlorhydria, and increased iron requirements during growth periods.[69] Iron deficiency anemia is the final stage of long-term iron deficiency.[69] Individuals at risk for iron deficiency include women of childbearing age with heavy menstrual periods, pregnant women, toddlers and preschool-age children due to increased iron requirements, premature infants with decreased iron stores at birth, and individuals with gastrointestinal disorders such as celiac disease and inflammatory bowel disease.[76,77] A number of different laboratory tests can be used to diagnose iron deficiency including serum ferritin, serum iron, percent transferrin saturation, and total iron-binding capacity (TIBC).[76] Characteristics of these tests are presented in Table 4. For this study, serum ferritin was selected as the indicator of iron status because it is a sensitive indicator of iron deficiency, an excellent indicator of iron stores, and can identify early-stage iron depletion.[78-80]

Table 4: Characteristics of laboratory tests used to diagnose iron deficiency

Test	Description	Criteria for diagnosing iron deficiency	Comments
Serum ferritin	Major iron storage protein. Serum level is an indicator of available body iron stores.[55]	<15 ng/mL, adult females[79,81]	It is a sensitive indicator of iron deficiency and can identify early negative iron status.[79,82] Ferritin is an positive acute-phase reactant protein produced by the liver and serum levels increase with inflammation.[55]
Serum iron	Measures the amount of ferric iron bound to transferrin in the blood.[83]	<60 µg/dl, adult females[55]	It is a good indicator of the amount of iron bound to transferrin.[82] It is not a sensitive measure of iron deficiency and is not useful in diagnosing early negative iron balance.[82,83]
Percent transferrin saturation	Transferrin is a protein that binds and carries iron in the blood.[55] This test measures the percentage of transferrin in the blood saturated with iron.[55]	<15%, adult females[55]	Percent transferrin saturation decreases with iron deficiency.[55] Transferrin is a negative acute-phase reactant protein produced by the liver. Serum levels decrease with inflammation.[55]
Total iron-binding capacity (TIBC)	Measures all iron-binding protein in the blood including transferrin.[55]	>400 µg/dl, females and males[84]	Serum transferrin levels increase with iron deficiency which increases TIBC.[55] TIBC is an indirect measure of serum transferrin and decreases with inflammation.[55,82] TIBC also decreases with liver disease and pernicious anemia caused by vitamin B_{12} deficiency.[55] TIBC is primarily an indicator of liver function.[82]

Based on data from NHANES, the average intake of iron (including iron from supplements) for women age 20 years and older is 17.3 mg per day, compared to the RDA of 18 mg/day.[85] Dietary sources of iron include heme iron found in meat, poultry, and fish and non-heme iron found in legumes, nuts, vegetables, and fortified grains.[76] Heme iron is absorbed much better than non-heme iron.[76] Once heme iron is separated from globin by proteases in the stomach and small intestine, it is readily absorbed in the proximal small intestine.[84] Non-heme iron must first be hydrolyzed from foods by HCl and proteases produced in the stomach and small intestine.[84] The freed iron is mostly in the ferric form which is insoluble and must be reduced to the ferrous form by HCl in the stomach or reductases produced in the duodenum to be absorbed.[84] Some of the ferric iron forms and insoluble complex in the alkaline environment of the duodenum, which decreases absorption.[84]

The prevalence of iron deficiency is estimated to be 13.2% for women aged 20-49 years in the U.S.[79] Racial differences can be seen with 19.9% of non-Hispanic black females having iron deficiency compared to 11.3% of non-Hispanic white females.[79]

1.1.5.2 Bariatric surgery and iron deficiency

Iron deficiency and iron deficiency anemia are common consequences of bariatric surgery.[10,62,63,86,87] The mechanisms resulting in iron deficiency after bariatric surgery include decreased intake of heme iron due to intolerance to meat, hypochlorhydria resulting in decreased iron absorption, and decreased intestinal absorption of iron after surgeries that bypass the duodenum, such as the RYGB.[9,24,65]

1.2 SPECIFIC AIMS AND HYPOTHESES

Due to the risk of developing folate and iron deficiency after bariatric surgery and the serious potential consequences to a fetus caused by these deficiencies, the primary goal of this study is to determine the association between bariatric surgery, specifically RYGB and LAGB, and the change in serum folate and serum ferritin from before surgery to one year following bariatric surgery among women of childbearing age. While there is research available in the literature on the prevalence of nutrient deficiencies following bariatric surgery, studies often have small sample sizes and inconsistent results. The specific aims of this research are:

Specific Aim 1: Conduct a review of the literature regarding the prevalence of postoperative deficiencies of folate and iron and their associations between bariatric surgery (RYGB, LAGB, and SG).

Specific Aim 2: Among women of childbearing age, estimate the prevalence of folate and iron deficiencies and examine the distributions of serum folate and ferritin levels prior to, and one year following, bariatric surgery. Determine the change in serum folate and serum ferritin levels from baseline to one year following bariatric surgery by type of bariatric surgery - RYGB vs. LAGB and determine if the changes in serum folate and serum ferritin differ by type of bariatric surgery. The hypothesis for this aim is women of childbearing age who have undergone RYGB surgery will have a larger decrease in serum folate and ferritin from baseline to one year following surgery compared to those women who had LAGB surgery.

Specific Aim 3: Identify factors associated with change in serum folate and serum ferritin levels from baseline to one year after RYGB among women of childbearing age. The hypothesis for this aim is lack of micronutrient supplement use, greater percent weight change, and alcohol consumption (folate only) are significantly associated with change in serum folate and serum ferritin levels among women of childbearing age who have undergone RYGB.

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

The objective of this literature review is to describe what has been published regarding the association between bariatric surgery and the prevalence of postoperative deficiencies of folate and iron. The literature review focuses on studies that included preoperative and postoperative data on folate or iron deficiency for the three most common types of bariatric surgery performed in the U.S. – SG, RYGB, and LAGB. While the population of interest for this study is women of childbearing age, there is a paucity of studies conducted solely in women or that provided results stratified by sex. Therefore, the literature review was expanded to include both adult women and men.

2.2 METHODS

2.2.1 Selection criteria for studies

The population of interest for the literature search includes adults who had undergone a RYGB, LAGB, or SG. The outcomes of interest are prevalence of folate deficiency, as determined by serum folate or RBC folate level, and iron deficiency, as determined by serum ferritin, serum iron, or transferrin saturation. Inclusion criteria for studies were: (1) age 18 years and older, (2) subjects had undergone a RYGB, LAGB, or SG, (3) reported prevalence of folate or iron deficiency both preoperatively and postoperatively one to two years following surgery, and (4) at least $\geq$ 40 subjects per surgical group. Studies were excluded if: (1) no females were included, (2) subjects were pregnant at the time of the study, or (3) the surgery was a revision of a previous bariatric surgery or a conversion to another bariatric surgery.

2.2.2 Search strategy

The OVID database was used to identify studies to be included in the literature review. The search strategy and results are presented in Figure 5. The final literature search was conducted on August 28, 2016. Articles were limited to English language and human studies only. No date restriction was used. For research design, the search was limited to clinical trials and observational studies. The following search terms were used to identify the exposure: *bariatric surgery OR bariatric surger* OR bariatric surgical OR metabolic surger* OR stomach stapling OR Roux-en-Y gastric bypass OR gastric bypass OR gastroplasty OR gastric band OR gastric banding OR laparoscopic adjustable gastric band OR sleeve gastrectomy OR gastric sleeve OR laparoscopic sleeve gastrectomy OR (gastroenterostomy AND obesity).* The search terms used for the outcomes were: *folate OR folic acid OR ferritin OR iron OR anemia, iron-deficiency OR vitamins OR vitamin* OR minerals OR micronutrients OR micronutrient* OR nutrient OR nutritional status OR deficiency diseases OR folic acid deficiency OR deficiency OR deficiencies OR malnutrition.* The search terms for the exposure and the outcomes were then combined using "AND", which resulted in 114 journal articles after removing duplicates. After screening the abstracts using the inclusion and exclusion criteria, 84 studies were excluded. An additional 12

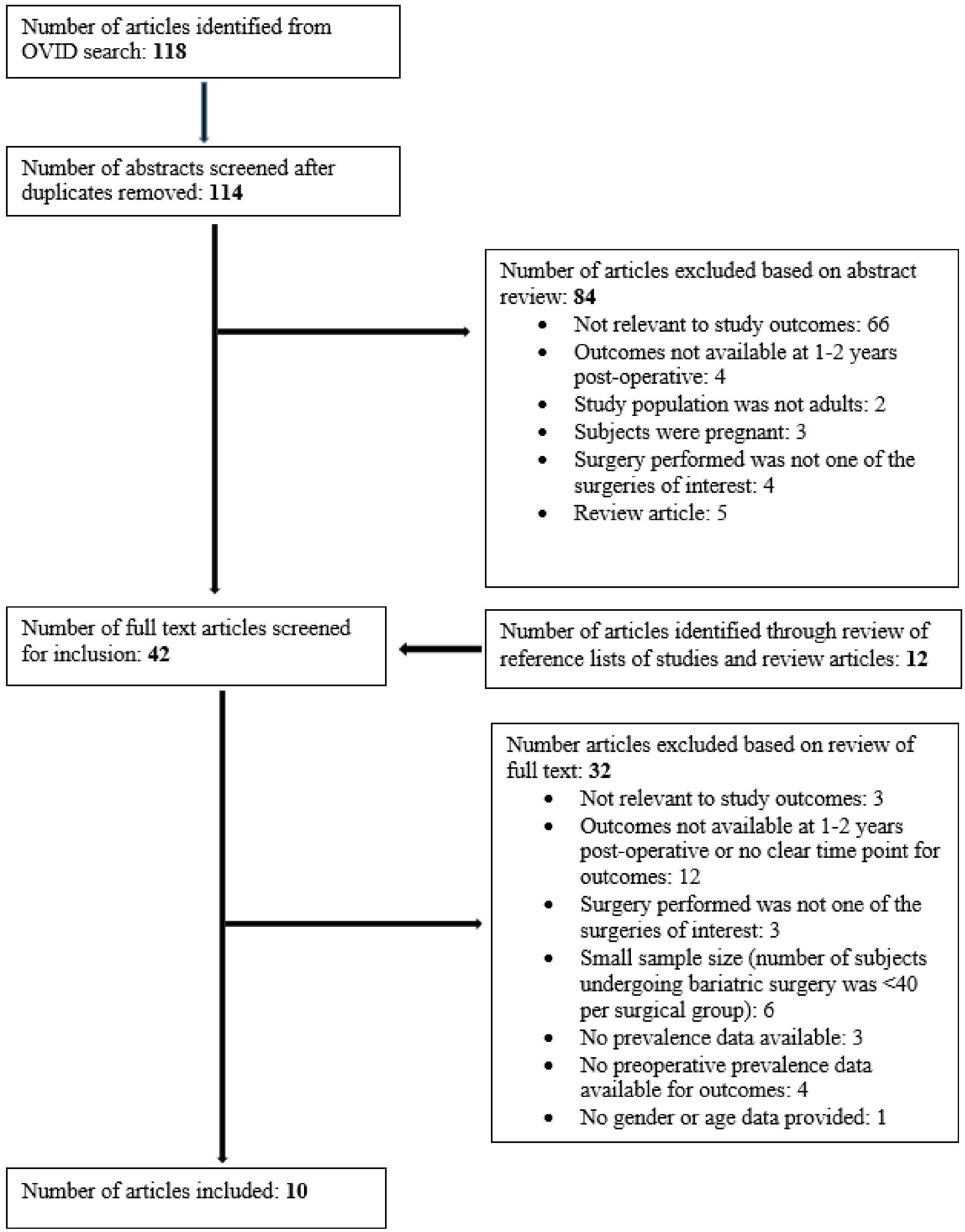


Figure 5: Flowchart of the literature review selection process

studies were identified by reviewing the reference lists of studies and review articles. Of the 42 full articles reviewed, 10 met the inclusion criteria and will be discussed in the literature review.

2.3 FOLATE AND IRON DEFICIENCY FOLLOWING BARIATRIC SURGERY

In this section, studies that compared preoperative and postoperative prevalence of folate or iron deficiency and met the inclusion/exclusion criteria for this review will be discussed. A summary of the main characteristics and results of the studies is provided in Table 5.

2.3.1 Folate or iron deficiency following sleeve gastrectomy

Vage et al.[88] evaluated changes in micronutrient levels after LSG in a group of 117 subjects (87 women, 30 men, mean age 40.3 years). The prevalence of folate deficiency, based on serum folate $\leq$5 nmol/L (equivalent to <2.21 ng/mL), was 7.5% at baseline and 8.0% 12 months after surgery. The prevalence of iron deficiency, based on serum ferritin level <25 µg/L (equivalent to <25 ng/mL), increased from 11.2% prior to surgery to 19.8% twelve months after LSG (p=.02). Mean serum levels were not reported for either micronutrient. All subjects were advised to take one MVI daily. At the 12-month follow-up, 74% of subjects were taking a MVI, 22% were taking a folate supplement, and 3% were taking an iron supplement. Vage et al.[88] reported there was no difference in vitamin or mineral status between those subjects who used supplements versus those who did not (p-value not reported).

2.3.2 Folate or iron deficiency following Roux-en-Y gastric bypass

2.3.2.1 Folate and iron deficiency

In a study by Blume et al.[89] of 170 patients (136 women, 34 men, mean age 39.5 years), the prevalence of folate deficiency, defined as serum folate <2.8 ng/mL, decreased from 6.5% prior to RYGB to 1.2% twelve months after surgery (statistical significance not reported) and then decreased to 0.6% at 36 months after surgery ($p=.009$, compared to baseline). Median serum folate levels increased from 8.1 ng/mL at baseline to 13.3 ng/mL at 12 months following surgery ($p<.05$) and increased to 14.6 ng/mL at 36 months ($p<.001$, compared to baseline level). Prevalence of iron deficiency, based on serum ferritin <10 ng/mL, was 5.3% prior to surgery and 4.1% twelve months following RYGB (statistical significance was not reported). Median serum ferritin levels decreased from 94.5 ng/mL prior to surgery to 69.6 ng/mL at 12 months after RYGB. The researchers reported that 12 months after RYGB, 74.7% of patients were taking a combination of individual vitamin or mineral supplements (MVI, iron, folic acid, or vitamin B_{12}).

Dogan et al.[90] conducted a clinical trial to compare the prevalence of a number of micronutrient deficiencies, including folate and iron, one year following RYGB, between subjects randomized to a standard MVI with minerals supplement or a specialized supplement designed for RYGB patients. The standard MVI with minerals supplement provided the RDA for folate and iron, whereas, the specialized MVI with minerals supplement provided three times the RDA for folate and five times the RDA for iron. Each supplement was to be taken once per day. There were 148 subjects with 74 per group. Sample size calculations were performed to provide 90% power and 95% sensitivity. The standard MVI with minerals group had 51 women and 23 men with an average age of 43.4 years, and the specialized MVI with minerals group had

53 women and 21 men with an average age of 45.3 years. Prior to surgery, 1.4% of subjects in the standard supplement group were folate deficient, based on serum folate <9 nmol/L (equivalent to <3.97 ng/mL), compared to 6.8% twelve months after RYGB (statistical significance not reported). Based on serum ferritin <20 µg/L (equivalent to <20 ng/mL), 6.7% of subjects in the standard supplement group had iron deficiency at baseline compared to 10.7% one year after RYGB (statistical significance not reported). The prevalence of iron deficiency was lower in the specialized supplement group twelve months after RYGB compared to the standard supplement group, with 1.3% of subjects in the specialized supplement group having iron deficiency (p=.03). There was no significant difference in the prevalence of folate deficiency between the two supplement groups.

2.3.2.2 Folate deficiency only

In a study of 58 subjects (46 women, 12 men, mean age 42 years) by Donadelli et al.[91], mean serum folate levels increased from 7.7 ng/mL at baseline to 14.1 ng/mL one year following RYGB (p<.05). The prevalence of folate deficiency, defined as serum folate <3 ng/mL, was 7.0% at baseline and 3.4% one year after RYGB (statistical significance not reported). All patients received the same commercial MVI and mineral supplement; however, data on compliance were not reported.

2.3.2.3 Iron deficiency only

Ikramuddin et al.[92] studied a number of outcomes, including prevalence of iron deficiency, in a group of 60 subjects (38 women, 22 men, mean age 49 years) one and two years following RYGB. Based on serum ferritin level (criteria to define deficiency not provided), the prevalence of iron deficiency was 2.0% prior to surgery, 14.0% at one year after RYGB, and 20.0% two years after RYGB (statistical significance not reported). Actual mean serum levels were not reported. Ikramuddin et al.[92] reported that iron deficiency was more common in women after RYGB, but specific data were not provided. Per the authors, all subjects who underwent RYGB were prescribed a MVI and other supplements, including iron. However, monitoring actual usage of prescribed supplements was not a part of the study protocol.

Bavaresco et al.[87] reported the prevalence of iron deficiency, based on serum iron <40 µg/dl, was 12.2% prior to surgery and 14.6% one year after RYBG (statistical significance was not reported) in a study of 48 patients (41 women, 7 men, mean age 41.9 years). The patients were instructed to take vitamin and mineral supplements during the postoperative period and compliance was monitored at follow-up visits but data on compliance were not reported.

2.3.3 Folate or iron deficiency following laparoscopic adjustable gastric banding, sleeve gastrectomy, and Roux-en-Y gastric bypass – studies with multiple surgery groups

Toh et al.[93] conducted a study to determine the prevalence of a number of micronutrient deficiencies, including folate and iron, in a group of 232 subjects (149 women, 83 men, mean age 46 years) prior to and one year after LSG, RYGB, and LAGB. All subjects, regardless of type of surgery, were instructed to take a liquid MVI and mineral supplement daily after surgery. In addition, RYGB patients were instructed to take a calcium supplement, vitamin B12 injections, and iron supplements, if they were determined to be deficient based on serum testing.

Results were available for 149 patients (103 RYGB, 46 LSG) one year after surgery. Due to a lack of postoperative biochemistry results for the subjects who underwent LAGB, results were only reported for the RYGB and LSG subjects. For subjects in the RYGB group, the prevalence of folate deficiency, defined as RBC folate <776 nmol/L, increased significantly from the preoperative period to one year after RYGB with levels of 1.0% vs. 12.0%, respectively ($p<.01$). Mean RBC folate level decreased from 1616 nmol/L prior to surgery to 1217 nmol/L one-year postoperative ($p<.01$). The prevalence of iron deficiency, defined as serum ferritin <15 µg/L (equivalent to <15 ng/mL), also increased significantly from 2.0% prior to surgery to 15.0% at one year after surgery ($p<.01$), and the mean serum ferritin level decreased from 170 µg/L at baseline to 117 µg/L one year following surgery ($p<.01$). In the LSG group, 7.0% of the subjects were folate deficient preoperatively, but no subjects were deficient one year following surgery. Mean RBC folate levels were 1582 nmol/L and 1504 nmol/L at baseline and one-year after LSG, respectively. The difference in prevalence of folate deficiency and change in mean RBC folate levels were not statistically significant. None of the subjects were iron deficient prior to surgery or one year following LSG. However, serum ferritin levels did decrease from 152 µg/L at baseline to 143 µg/L one year after surgery ($p<.05$).

Coupaye et al.[94] studied the prevalence of micronutrient deficiencies one year after LAGB and RYGB. Of the 70 subjects, 21 had undergone LAGB (18 women, 3 men, mean age 35 years) and 49 had undergone RYGB (45 women, 4 men, mean age 43 years). All patients who underwent RYGB were prescribed a MVI supplement, which also contained calcium and iron, as well as intramuscular vitamin B_{12} supplementation.

For the LAGB group, the prevalence of folate deficiency, defined as serum folate <3 µg/L (equivalent to <3 ng/mL), was 5.0% prior to surgery and 10.0% one year after surgery; however, this change was not statistically different. The mean serum folate levels in the LAGB group were 7.1 µg/L at baseline and 5.7 µg/L one year after surgery. This decrease was not statistically significant. In contrast, mean serum folate levels increased significantly ($p<.001$) from 6.1 µg/L prior to surgery to 17.6 µg/L one year after RYGB. The difference in the prevalence of folate deficiency, 4.0% prior to surgery and 0% one year after RYGB, was not significant. The prevalence of iron deficiency, based on serum ferritin <3 µg/L (equivalent to <3 ng/mL), was 15.0% before surgery and 5.0% after LAGB. This change in prevalence was not significant. Mean serum ferritin levels in the LABG group were similar at both time points (86.3 µg/L prior to surgery vs. 86.8 µg/L one year after surgery). In the RYGB group, the prevalence of iron deficiency was similar at both time points with 2.0% of subjects deficient in iron prior to surgery and 4.0% after RYGB. The mean serum ferritin levels were 96.4 µg/L at baseline and 69.8 µg/L one year after surgery; however, this change was not significant.

In a smaller study of 86 subjects, Coupaye et al.[95] compared the prevalence of folate and iron deficiency one year after LSG and RYGB. The subjects were matched for age, gender, and weight six months after surgery. There were 43 subjects in the LSG group (31 women, 12 men, mean age 45 years) and 43 subjects in the RYGB group (31 women, 12 men, mean age 44 years). Multivitamin supplements, also containing the minerals iron and calcium, were provided to patients with deficiencies identified prior to surgery. After surgery, this MVI and mineral supplement was prescribed for all patients. In the LSG group, the prevalence of folate deficiency, defined as serum folate <3 µg/L, remained constant with 7.0% of subjects deficient prior to surgery and one year following surgery. Serum folate levels increased significantly from 6.6 µg/L at baseline to 11.0 µg/L one year after LSG ($p<.001$). For the RYGB group, the prevalence of folate deficiency was 7.0% at baseline and 3.0% one year after surgery; however, this change in prevalence was not significant. Similar to the LSG group, serum mean serum folate levels increased from 6.2 µg/L at baseline to 14.0 µg/L one-year after RYGB ($p<.001$). Coupaye et al.[95] obtained both serum ferritin and transferrin saturation levels, but used transferrin saturation of <20% to determine the prevalence of iron deficiency. The prevalence of iron deficiency in the LSG group decreased significantly from 53.0% of subjects prior to surgery to 30.0% one year after LSG ($p<.05$). Mean transferrin saturation increased from 19.3% to 24.8% ($p<.01$). The change in mean serum ferritin from 168.7 µg/L at baseline to 184.4 µg/L one year after LSG was not statistically significant. Prevalence of iron deficiency also decreased in the RYGB group with 49.0% of subjects deficient at baseline compared to 20.0% of subjects one year after surgery ($p<.01$). In the RYGB group, mean transferrin saturation increased from 19.8% at baseline to 25.1% one-year following surgery ($p<.01$). The mean serum ferritin levels, 134.8 µg/L prior to surgery and 97.3 µg/L after surgery, were not significantly different.

2.3.4 Summary of findings

2.3.4.1 Folate deficiency after bariatric surgery

The prevalence of folate deficiency one year after RYGB ranged from 0% - 12.0% in six studies.[89-91,93-95] Of these studies, only Toh et al.[93] reported a significant increase in the prevalence of folate deficiency from baseline to one year following RYGB. Three studies[89-91] did not report statistical significance of difference in prevalence. For the three LSG studies[88,93,95], the prevalence of folate deficiency one year after surgery ranged from 0% - 8.0% and none reported a statistically significant difference in the prevalence of folate from baseline to one year following surgery. For LAGB, Coupaye et al.[94] reported that the prevalence of deficiency one year after surgery was not significantly different from baseline.

2.3.4.2 Iron deficiency after bariatric surgery

Seven studies[87,89,90,92-95] compared the preoperative and one-year postoperative prevalence of iron deficiency after RYGB. The prevalence of iron deficiency one year following RYGB in these studies ranged from 4.0% - 20.0%. Of these studies, only Toh et al.[93] reported a significant increase in the prevalence of iron deficiency from baseline to one year following RYGB. In contrast, Coupaye et al.[95] reported that the prevalence of iron deficiency decreased significantly from baseline to one year after RYGB. The other three studies[87,90,92] did not report statistical significance of differences. Three studies[88,93,95] compared baseline to one-year postoperative prevalence of iron deficiency after LSG. Vage et al.[88] reported a significant increase in the prevalence of iron deficiency one year after LSG; while Coupaye et al.[95] reported a significant decrease. Toh et al.[93] found no significant difference in the prevalence of iron deficiency at the two time points. Only one study[94] investigated the prevalence of iron deficiency prior to and one year after LAGB, and the difference in prevalence of deficiency was not statistically significant.

2.3.4.3 Study characteristics

The number of subjects ranged from 48 – 170, with a median of 74 subjects. All of the studies had a larger percentage of women compared to men; however, none of the studies stratified the results for folate or iron deficiency by sex. The mean age of the subjects ranged from 35 – 49 years. Researchers measured serum folate levels to diagnose folate deficiency, except for Toh et al.[93] who used RBC folate levels. The serum biomarker used to identify iron deficiency varied between studies. Six studies[88-90,92-94] used serum ferritin, one study [87] used serum iron, and one study[95] used transferrin saturation. As presented previously in Table 4, these biomarkers vary in what is measured, the phase of iron deficiency detected, and other factors that can cause a decreased serum value. Another factor that varied between studies was the laboratory cutoffs used to define folate and ferritin deficiency. The definition of folate deficiency based on serum folate ranged from 2.21 – 3.97 ng/mL. For studies that used serum ferritin to identify iron deficiency, the criteria ranged from 3 – 25 ng/mL. Studies that used a serum level on the low end of the range (e.g. Coupaye et al.[94]) would classify less individuals as deficient based on the stricter criteria compared to a study that used a higher serum ferritin level (e.g. Vage et al.[88]). This could explain some of the difference in the prevalence of deficiency.

Table 5: Summary of studies comparing preoperative and postoperative prevalence of folate or iron deficiency

Author, year (Location)	Surgery	Baseline sample size, gender, mean age (y)	Indicator of deficiency (criteria for defining deficiency)	Recommended supplementation post-op	Prevalence of folate deficiency: baseline vs. 1-year post-op (p-value)	Prevalence of iron deficiency: baseline vs. 1-year post-op (p-value)
Vage, 2014[88] (Norway)	LSG	117 subjects (87 women, 30 men, 40.3 y)	Serum folate ($\leq$5 nmol/L equivalent to <2.21 ng/mL) Serum ferritin (<25 µg/L equivalent to <25 ng/mL)	MVI daily; 74% of subjects reported taking MVI daily at 12-month follow-up	7.5% vs. 8.0% (p=1.0, NS)	↑ 11.2% vs. 19.8% (p=.02)
Bavaresco, 2010[87] (Brazil)	RYGB	48 subjects (41 women, 7 men, 41.9 y)	Serum iron (<40 µg/dL)	Vitamin and mineral supplementation daily (specific information NR)	N/A	12.2% vs. 14.6 % (NR)
Blume, 2012[89] (Brazil)	RYGB	170 subjects (136 women, 34 men 39.5 y)	Serum folate (<2.8 ng/mL) Serum ferritin (<10 ng/mL)	72% of subjects reported taking a combination of individual vitamin or mineral supplements (MVI, iron, folic acid, or vitamin B_{12})	6.5% vs. 1.2% (NR)	5.3% vs. 4.1% (NR)
Donadelli, 2012[91] (Brazil)	RYGB	58 subjects (46 women, 12 men, 42 y)	Serum folate (<3 ng/mL)	MVI with minerals daily	7.0% vs. 3.4% (NR)	N/A
Dogan, 2014[90] (The Netherlands)	RYGB	74 subjects[a] (51 women, 23 men, 43.4 y)	Serum folate (<9 nmol/L equivalent to 3.97 ng/mL) Serum ferritin (<20 µg/L equivalent to <20 ng/mL)	MVI with minerals including iron daily	1.4% vs. 6.8% (NR)	6.7% vs. 10.7% (NR)
Ikramuddin, 2015[92] (U.S. and Taiwan)	RYGB	60 subjects (38 women, 22 men, 49 y)	Serum ferritin (NR)	MVI and iron supplement daily	N/A	2.0% vs. 14.0% (NR)

Abbreviations: y: years; post-op: post-operative; LSG: laparoscopic sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding; MVI: multivitamin; NR: not reported; NS: not statistically significant; N/A: not applicable for the individual study
Symbols: ↑: prevalence of deficiency increased significantly from baseline to 1-year post-op
[a]Reporting only data from the standard multivitamin with minerals supplement group.

Table 5 (continued): Summary of studies comparing preoperative and postoperative prevalence of folate or iron deficiency

Author, year (Location)	Surgery	Baseline sample size, gender, mean age (y)	Indicator of deficiency (criteria for defining deficiency)	Recommended supplementation post-op	Prevalence of folate deficiency: baseline vs. 1-year post-op (p-value)	Prevalence of iron deficiency: baseline vs. 1-year post-op (p-value)
Toh, 2009[93b] (Australia)	LSG	46 (NR, NR)	RBC folate (<776 nmol/L) Serum ferritin (<15 µg/L equivalent to <15 ng/mL)	MVI with minerals daily	7.0% vs. 0% (NS)	0% vs. 0%
	RYGB	103 (NR, NR)		MVI with minerals daily	↑ 1.0% vs. 12.0% (p<.01)	↑ 2.0% vs. 15.0% (p<.01)
Coupaye, 2009[94] (France)	LAGB	21 (18 women, 3 men, 35 y)	Serum folate (<3 µg/L equivalent to <3 ng/mL) Serum ferritin (<3 µg/L equivalent to <3 ng/mL)	None	5.0% vs. 10.0% (NS)	15.0% vs. 5.0% (NS)
	RYGB	49 (45 women, 4 men, 43 y)		MVI containing iron and calcium daily; intramuscular vitamin B_{12}	4.0% vs. 0% (NS)	2.0% vs. 4.0% (NS)
Coupaye, 2014[95] (France)	LSG	43 (31 women, 12 men, 45 y)	Serum folate (<3 µg/L equivalent to <3 ng/mL) Transferrin saturation (<20%)	MVI containing iron and calcium daily	7.0% vs. 7.0%	↓ 53.0% vs. 30.0% (p<.05)
	RYGB	43 (31 women, 12 men, 44 y)		MVI containing iron and calcium daily	7.0% vs. 3.0% (NS)	RYGB: ↓ 49.0% vs. 20.0% (p<.01)

Abbreviations: y: years; post-op: post-operative; LSG: laparoscopic sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding; RBC: red blood cell; NR: not reported; NS: not statistically significant; N/A: not applicable for the individual study

Symbols: ↑: prevalence of deficiency increased significantly from baseline to 1-year post-op; ↓: prevalence of deficiency decreased significantly from baseline to 1-year post-op

[b]232 subjects (149 women, 83 men, 46 y) at baseline. Results available for 149 subjects (sex and mean age NR).

2.4 FOLATE AND IRON DEFICIENCY - COMPARISON BETWEEN BARIATRIC SURGERIES

Three studies compared the prevalence of micronutrient deficiencies, including folate and iron, between different types of bariatric surgeries met the inclusion/exclusion criteria for this review. Two of the studies, Coupaye et al.[94] and Coupaye et al.[95], also compared preoperative and postoperative prevalence of deficiency and are included in both the prior section and this section for discussion. Table 6 provides a summary of the main characteristics and results of these studies.

2.4.1 Laparoscopic adjustable gastric banding versus Roux-en-Y gastric bypass

Coupaye et al.[94] compared the prevalence of folate and iron deficiency one year after LAGB and RYGB (the details of the study were described previously in section 2.3.3). Mean serum folate level was significantly lower following LAGB (5.7 µg/L) than RYGB (17.6 µg/L) ($p<.001$), and there was a significantly higher prevalence of folate deficiency at one year after LABG (10.0%) when compared to RYGB (0%) ($p<.05$). Mean serum ferritin level one year after RYGB was 69.8 µg/L compared to 86.8 µg/L after LAGB; however, this difference was not significant. The prevalence of iron deficiency one year after LAGB was 5.0% compared to 4.0% after RYGB, which was not statistically different.

2.4.2 Sleeve gastrectomy versus Roux-en-Y gastric bypass

In another study, Coupaye et al.[95] compared the prevalence of folate and iron deficiency one year after LSG and RYGB (the details of the study were described in section 2.3.3). Mean serum folate levels were not statistically different one year after LSG and RYGB, with values of 11.0 µg/L vs. 14.0 µg/L, respectively. The researchers reported the prevalence of folate deficiency one year following surgery, 7.0% for LSG vs. 3.0% for RYGB, was not statistically different. The prevalence of iron deficiency, based on transferrin saturation, was 30.0% one year after LSG and 20.0% after RYGB; however, the difference was not statistically significant. Similarly, mean transferrin saturation was not statistically different between the two surgeries one year after surgery, with values of 24.8% for the LSG group and 25.1% for the RYGB group. However, mean serum ferritin level was significantly lower ($p<.05$) at one year following RYGB when compared to one year after LSG (97.3 µg/L vs. 184.4 µg/L, respectively). The researchers noted one year following surgery, 93% of patients in the RYGB group were taking the recommended MVI supplement compared to 53% of LSG patients ($p<.01$).

Gehrer et al.[63] compared the rates of micronutrient deficiencies over a 36-month time period following bariatric surgery for subjects who had a LSG or a laparoscopic RYGB. The study had 136 subjects, with 50 subjects (37 women, 13 men, mean age 41.9 years) in the LSG group and 86 subjects (61 women, 25 men, mean age 43.5 years) in the RYGB group. All subjects were prescribed a daily MVI supplement, which also included calcium, magnesium, and zinc, to be taken postoperatively. Folate deficiency was defined by serum folate $\leq$ 6 nmol/L (equivalent to $\leq$2.65 ng/mL), and serum ferritin $\leq$ 30 ng/mL indicated iron deficiency. Mean serum levels were not reported. One year after surgery, the prevalence of folate deficiency in the LSG group was 16.0% compared to 9.0% in the RYGB group. The prevalence of iron deficiency was 16.0% one year after LSG and 24.0% after RYGB. Statistical testing for significance of between-group differences was not reported at one year.

2.4.3 Summary of findings between surgeries

Only three studies were identified that compared the prevalence of folate or iron deficiency between the bariatric surgeries of interest one year after surgery and met the inclusion/exclusion criteria for this review. One study[94] compared results for LAGB and RYGB. The prevalence of folate deficiency was significantly greater after LAGB when compared to RYGB. The prevalence of iron deficiency was similar for both surgery groups. Coupaye et al.[94] did not control or adjust for potential confounding. The authors did not report sample size calculations or power level; however, based on the small sample size the study may have been underpowered.

Two studies[63,95] compared the prevalence of folate and iron deficiencies one year after surgery for subjects who underwent LSG and RYGB. Coupaye et al.[95] did not find a statistically significant difference in the prevalence of folate deficiency or iron deficiency between surgeries at one year after surgery. The subjects were matched for age, gender, and weight six months after surgery to control for potential confounding. Coupaye et al.[94] noted their study lacked power for statistical comparisons; however, the power level for the study was not reported. Gehrer et al.[63] did not report statistical testing at the one-year time point after surgery for either nutrient deficiency. Gehrer et al.[63] did not control or adjust for potential confounding. The authors did state the LSG and RYGB groups were similar in age, preoperative BMI, and gender.

The number of subjects ranged from 70 - 136. The mean age of the subjects ranged from 35 – 45 years. All three studies[63,94,95] used serum folate levels to diagnose folate deficiency and similar criteria to define deficiency. For identification of iron deficiency, one study[95] used transferrin saturation and two studies[63,94] used serum ferritin. Coupaye et al.[94] defined deficiency as serum ferritin <3 ng/mL while Gehrer et al.[63] defined deficiency as ≤30 ng/mL. This difference in criteria used could explain, in part, the lower prevalence of iron deficiency identified one year after surgery by Coupaye et al.[94] (5.0% after LAGB and 4.0% after RYGB) compared to Gehrer et al.[63] (16.0% after LSG and 24.0% after RYGB).

Table 6: Summary of studies comparing the prevalence of folate and iron deficiency one year post-surgery between bariatric surgeries

Author, year (Location)	Surgery	Baseline total and group sample sizes, women vs. men, mean age (y)	Indicator of deficiency (criteria for defining deficiency)	Recommended supplementation post-op	Prevalence of folate deficiency at 1-year post-op (p-value for between surgery comparisons)	Prevalence of iron deficiency at 1-year post-op (p-value for between surgery comparisons)
Coupaye, 2009[94] (France)	LAGB vs. RYGB	70 subjects: 21 LAGB (18 women, 3 men, 35 y) 49 RYGB (45 women, 4 men, 43 y)	Serum folate (<3 µg/L equivalent to <3 ng/mL) Serum ferritin (<3 µg/L equivalent to <3 ng/mL)	LAGB: none RYGB: MVI containing iron and calcium daily; intramuscular vitamin B_{12}	LAGB: 10.0% RYGB: 0% LAGB > RYGB ($p<.05$)	LAGB: 5.0% RYGB: 4.0% (NS)
Coupaye, 2014[95] (France)	LSG vs. RYGB	86 subjects: 43 LSG (31 women, 12 men, 45 y) 43 RYGB (31 women, 12 men, 44 y)	Serum folate (<3 µg/L equivalent to <3 ng/mL) Transferrin saturation (<20%)	MVI containing iron and calcium daily	LSG: 7.0% RYGB: 3.0% (NS)	LSG: 30.0% RYGB: 20.0% (NS)
Gehrer, 2010[63] (Switzerland)	LSG vs. RYGB	136 subjects: 50 LSG (37 women, 13 men, 41.9 y) 86 RYGB (61 women, 25 men, 43.5 y)	Serum folate (≤ 6 nmol/L equivalent to <2.65 ng/mL) Serum ferritin (≤ 30 ng/mL)	MVI containing calcium, magnesium, and zinc daily	LSG: 16.0% RYGB: 9.0% (NR)	LSG: 16.0% RYGB: 24.0% (NR)

Abbreviations: y: years; post-op: post-operative; LAGB: laparoscopic adjustable gastric banding; LSG: laparoscopic sleeve gastrectomy; RYGB: Roux-en-Y gastric bypass; MVI: multivitamin; NR: not reported; NS: not statistically significant

2.5 SUMMARY

2.5.1 Study quality and limitations

2.5.1.1 Sample size and power

A significant limitation to the majority of the studies was sample size. The number of subjects in the studies reported ranged from 48 – 170, with a median of 80 subjects. Studies with small sample sizes may not have had adequate power to detect meaningful differences in results as statistically significant. For example, the study by Coupaye et al.[94], which only had 70 subjects (21 in the LAGB group and 49 in the RYGB group), did find significant differences in the prevalence of folate or iron deficiency between baseline to one year after surgery for either group or for the prevalence of iron deficiency between the two surgery groups.

Only two studies included sample size calculations and estimated power. Dogan et al.[90] conducted sample size calculations to detect a 25% reduction in iron deficiency between study groups (standard MVI with minerals vs. specialized supplement) one year after surgery. The researchers determined 75 subjects were needed per group, for a total of 150 subjects, to provide 90% power. Two subjects were excluded after randomization. Included in the analyses were 148 subjects with 74 per group. Ikramuddin et al.[92] reported 90% power for their study of 120 subjects (60 per group). However, the sample size calculation was based on percent success in reduction of glycated hemoglobin, an indicator of glycemic control, between study groups (RYGB vs. lifestyle and medical management for obesity) which is not a variable relevant to this review.

2.5.1.2 Statistical testing

A common factor among the majority of the studies reviewed was a lack of testing for statistical significance between preoperative and postoperative prevalence of folate and iron deficiency or differences between surgery groups. Of the studies that met the inclusion/exclusion for this review, only four[88,93-95] out of ten reported statistical testing of differences in prevalence one year postoperatively. Inadequate statistical power may have been the rationale for the lack of statistical tests reported in some studies.

2.5.1.3 Definition of deficiencies

Researchers used different serum biomarkers to identify deficiencies. For folate deficiency, serum folate was used in all studies, except for Toh et al.[93] who used RBC folate levels. For identification of iron deficiency, seven studies[63,88-90,92-94] used serum ferritin, one study [87] used serum iron, and one study[95] used transferrin saturation. As presented previously in Table 3 and Table 4, these biomarkers vary in what is measured, the time period of deficiency identified, and other factors besides nutrient deficiency that can affect serum level.

In addition, there was variation in the laboratory cutoffs used to define folate and ferritin deficiency. The definition of folate deficiency using serum folate ranged from 2.21 – 3.97 ng/mL. For studies that used serum ferritin to identify iron deficiency, the criteria ranged from 3 – 30 ng/mL. The use of different serum levels to diagnose deficiency could account for some of the differences in prevalence of deficiency with studies using stricter criteria classifying less individuals as deficient compared to studies that used a higher serum level.

2.5.1.4 Vitamin and mineral supplement usage

Another major limitation was a lack of consistent data obtained on vitamin and mineral supplement usage after surgery. Recommendations for micronutrient supplementation varied between studies, particularly with respect to minerals. Only a few studies[88,89,95] reported data on the subjects' intake of vitamin and mineral supplements.

2.5.1.5 Stratification of results by sex

Another limitation of the reviewed studies is none of the studies stratified the results by sex. This is relevant to the proposed research because the population of interest is women of child-bearing age.

2.5.2 Need for additional research

While studies have been conducted to determine the prevalence of folate and iron deficiency after bariatric surgery, the results are conflicting and study quality has been an issue. Examining the results by procedure, there was no significant difference in the prevalence of folate or iron deficiency from baseline to one year after surgery for the one study[94] that looked at LAGB. None of the LSG studies[88,93,95] reported significant differences in preoperative vs. one-year postoperative prevalence of folate deficiency. For iron deficiency following LSG, one study[88] reported a significant increase in prevalence, one study[95] reported a significant decrease in prevalence, and one study[93] reported no significant difference in the prevalence of iron deficiency from baseline to one-year following surgery. One study[93] reported a significant increase in the prevalence of folate deficiency from baseline to one year after RYGB; while, two studies[94,95] reported no significant differences. The prevalence of iron deficiency after RYGB increased significantly in one study[93], decreased significantly in another study[95], and did change significantly from baseline to one-year following surgery in one study[94]. For between surgery comparisons, the prevalence of folate deficiency was significantly higher one year following LAGB compared to RYGB in one study[94]. There was no significant difference in the prevalence of folate deficiency one year after surgery in a study[95] that compared LSG to RYGB. No significant differences were reported for the prevalence of iron deficiency one year after surgery in a study[94] comparing LAGB to RYGB or a study[95] comparing LSG to RYGB.

The proposed study will add to the existing research by addressing the limitations previously discussed. This study will have a larger sample size of 427 subjects, based on sample and effect size calculations. The prevalence of folate and iron deficiencies will be determined as well as the change in serum folate and ferritin levels from baseline to one-year after surgery. Also, between group comparisons by surgery type will be evaluated. Differences will be tested for statistical significance. Data on supplement intake will be included in the analysis. Finally, to address the aims of the study, the subjects will be limited to adult females of childbearing age only.

3.0 METHODOLOGY

3.1 EXPERIMENTAL DESIGN

This study involved analyzing stored serum samples from a cohort of childbearing-age women before and after having undergone bariatric surgery to determine the change in serum folate and serum ferritin levels. The serum samples were from adult females who participated in the Longitudinal Assessment of Bariatric Surgery (LABS) study. The proposed research was approved to be conducted as an ancillary study to the LABS study. The study was granted "exempt" status by the University of Pittsburgh Institutional Review Board on February 19, 2016.

LABS was a multicenter, observational study that collected data on candidates for bariatric surgery prior to and following surgery at ten clinical centers throughout the U.S.[4,96] LABS had three phases.[96] The goal of LABS-1 was to evaluate the short-term safety of bariatric surgery.[96] The LABS-1 cohort included 4776 participants with limited clinical data to assess adverse outcomes within 30 days after surgery.[97] LABS-2 focused on the longer-term safety and efficacy of bariatric surgery and collected substantial data on clinical and psychiatric/psychological factors including weight, comorbid conditions, and functional impairment.[4,96] LABS-3 comprised two "mechanistic" studies: a diabetes study and a psychosocial study.[98] The diabetes study investigated the physiological mechanisms involved in improved glycemic control after RYGB for subjects with type 2 diabetes mellitus.[98] The psychosocial study involved subsets of participants from LABS-2 and focused on psychosocial and behavioral aspects of obesity.[96,99]

3.2 SUBJECTS

The serum samples analyzed for this ancillary study were from a subset of 426 participants from LABS-2 who met all of the inclusion criteria and none of the exclusion criteria specified in section 3.2.2.

3.2.1 Description of the LABS-2 participants

LABS-2 involved 2458 participants who underwent bariatric surgery between February 2006 and April 2009.[100] Each participant underwent a first-time bariatric surgery performed by a LABS-certified surgeon at one of the following centers: University of Pittsburgh Medical Center (Pennsylvania), Columbia University Medical Center and Weill-Cornell University Medical Center (New York), University Health Systems of Eastern North Carolina and East Carolina University (North Carolina), Neuropsychiatric Research Institute (North Dakota), Oregon Health and Science University and Legacy Good Samaritan Hospital (Oregon), and Virginia Mason Medical Center and University of Washington (Washington).[96,100] Over two-thirds (70.7%) of participants underwent a RYGB. For the participants who underwent a RYGB, the surgery was performed laparoscopically in 88.6% of participants and performed as an open procedure in 11.4% of participants.[100] In addition, 24.8% of participants underwent LAGB, and 4.5% had another type of surgery such as SG or BPD with duodenal switch.[100] All participants were at least 18 years old.[96] The median age was 46 years with a range of 18 - 78 years.[100]

The LABS-2 cohort included 1931 female participants.[4] A summary of selected baseline characteristics for the female LABS-2 participants is presented in Table 7. Of these women, 50.9% were between the ages of 18-45 years at surgery.[4,5] Specifically for these women of childbearing age, 74.1% underwent a RYGB, 22.2% LAGB, and 1.9% SG.[4,5] While the most common bariatric surgical procedure currently performed in the U.S. is the SG[1], this was not the case in LABS which ended enrollment in 2009. Thus, due to the low number of LABS-2 participants who underwent this procedure, only RYGB and LAGB participants were included.

Table 7: Summary of selected baseline characteristics for all LABS-2 female participants (n = 1931)

Baseline Characteristic	**Results**
Age in years - median (quartiles)	45 (36, 54)
Childbearing age range of 18-45 years at surgery – n (%)	984 (50.9%)
Race – n (%):	
White	1623 (84.6%)
Black	224 (11.7%)
Multiple races	43 (2.2%)
Other	28 (1.5%)
Ethnicity – n (%):	
Hispanic	101 (5.2%)
Non-Hispanic	1829 (94.8%)
BMI in kg/m^2 – median (quartiles)	45.7 (41.6, 51.0)

Source: Adapted from Tables 1 and 2 of: Belle SH, Berk PD, Chapman WH, et al. Baseline characteristics of participants in the Longitudinal Assessment of Bariatric Surgery-2 (LABS-2) study. *Surg Obes Relat Dis.* 2013;9(6):926-935.[100]

3.2.2 Inclusion criteria

The subjects for this ancillary study were randomly selected by LABS DCC staff from the group of LABS-2 participants who met the following inclusion criteria:

- female participants of the LABS-2 study
- in the childbearing age range of 18-45 years at surgery
- had both preoperative and one-year postoperative serum samples stored at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Biospecimen Repository
- underwent either a RYGB (laparoscopic or open procedure) or a LAGB.

3.2.3 Exclusion criteria

Subjects were excluded from the study if:

- either the preoperative or one-year postoperative serum sample was hemolyzed.

For generalizability, the subjects were of varying races and ethnicities and from different LABS clinical centers. A visual representation of recruitment of participants for LABS-2 and flow to the current study is shown in Figure 6.

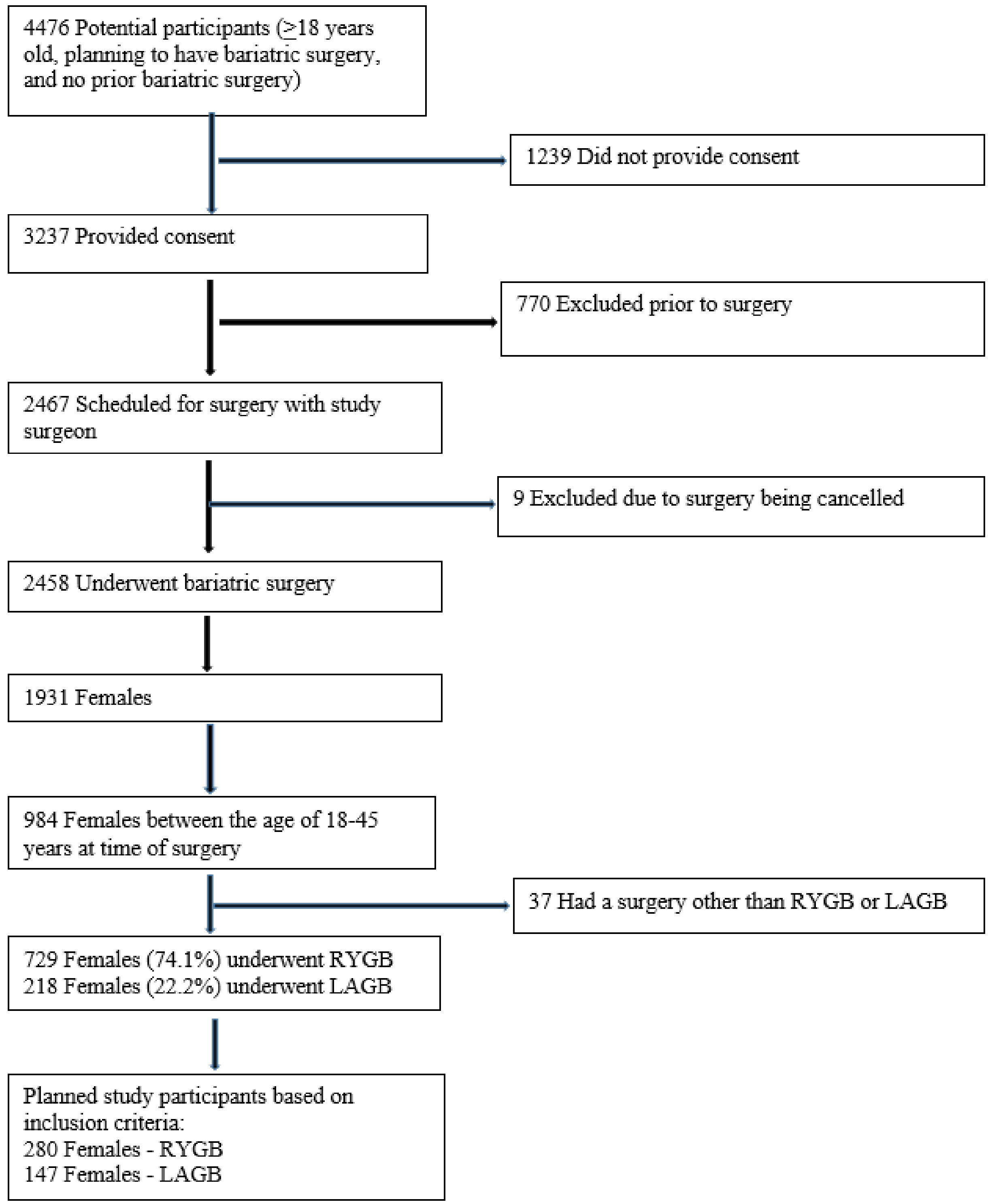


Figure 6: Flowchart of recruitment for LABS-2 and the current study.
Adapted from Figure 1 of Belle SH, Berk PD, Chapman WH, et al. Baseline characteristics of participants in the Longitudinal Assessment of Bariatric Surgery-2 (LABS-2) study. Surg Obes Relat Dis. 2013;9(6):926-935[100] and Figure of King WC, Chen J, Mitchell JE, et al. Prevalence of alcohol use disorders before and after bariatric surgery. JAMA. 2012;307(23):2516-2525[101].

3.3 SAMPLE SIZE AND POWER ANALYSIS

Sample size calculations were conducted to determine the number required to obtain estimates within 5% of the true prevalence of deficiency of folate and iron, individually, with a confidence level of 95%. Sample sizes were calculated using the formula by Daniel[102] and prevalence data from the study by Gehrer et al.[63] to estimate the true prevalence of folate and iron deficiency after LSG and RYGB. Due to a lack of available data on prevalence of folate and iron deficiency after LAGB, LSG data from the Gehrer et al.[63] study were used. Gehrer et al.[63] reported prevalence of 16% for folate deficiency and 16% for iron deficiency one year after LSG and 9% for folate deficiency and 24% for iron deficiency one year after RYGB. Based on these data, a sample size of 206 LAGB subjects was needed to obtain an estimate of the prevalence of folate deficiency and 206 subjects to obtain an estimate of the prevalence of iron deficiency one year after surgery with the precision noted above. For RYGB, 126 subjects were needed for the prevalence of folate deficiency and 280 subjects for the prevalence of iron deficiency one year after surgery with the precision noted above.

The largest sample size required for estimating prevalence was selected resulting in a target sample size of 280 RYGB and 280 LAGB subjects. The 280 RYGB subjects were a random sample of the 729 LABS-2 female participants who underwent RYGB and met the inclusion criteria for the study. However, there were only 218 LABS-2 female participants who underwent LAGB and of these participants only 147 met the inclusion criteria for the study with the age criteria being the limiting factor.

Since data collection for LABS-2 is complete, the sample size is fixed and funding for this ancillary study was limited, so, using the sample sizes derived above, effect size calculations were conducted to address the second aim of the study, which was to determine if the change in serum folate level and serum ferritin level from baseline to one-year following bariatric surgery for women of childbearing age differed by type of bariatric surgery - RYGB vs. LAGB. PASS version 14 (NCSS Statistical Software) was used to conduct the effect size calculations. With sample sizes of 280 RYGB subjects and 147 LAGB subjects, there was 80% power to reject the null hypothesis of equal means of folate or ferritin levels between subjects in each surgery group when the effect size is 0.3, with α <.05, using a two-sided two-sample equal-variance t-test. An effect size of 0.3 based on Cohen's *d* is considered "small" to "medium".[103]

For the third aim of the study, which was to identify factors associated with change in serum folate and serum ferritin levels from baseline to one year after RYGB among women of childbearing age, PASS version 14 (NCSS Statistical Software) was used to calculate the minimum R-squared of independent variables in a linear regression model that can be detected based on the sample size. With a sample size of 280 subjects, there was 80% power to detect an R-squared of 0.07 attributed to 20 independent variables using an F-Test with α <.05.

3.4 OUTCOME MEASURES AND ASSESSMENT

The primary outcome measures for the study were the change in serum folate and serum ferritin levels from baseline (preoperative) to one year after RYGB or LAGB. The secondary outcomes were the prevalence of folate and iron deficiencies one year after RYGB or LABG. Serum levels of both micronutrients were determined from each participant's serum sample following the methods described below. Both preoperative and one-year postoperative serum levels were determined for each subject. The serum analyses were conducted by staff at the Heinz Nutrition Laboratory at the University of Pittsburgh's Graduate School of Public Health under the guidance of the Lab Director, Joseph M. Zmuda, PhD, Associate Professor of Epidemiology and Beth Ann Hauth, Lab Manager. Per the request of the Lab Manager, both the preoperative and one-year postoperative serum sample for each subject were analyzed in the same batch of 30 samples. However, the serial identification numbers were masked so the lab technicians did not know which samples came from the same subject.

3.4.1 Determining folate status

Folate status was determined by the serum folate level measured using a microbiological assay provided by ALPCO (Salem, NH). For the folate analysis, 300 µL of serum was required. Diluted samples were added to microtiter plate wells coated with Lactobacillus rhamnosus. During incubation at 37°C for 48 hours, the growth of the bacteria is directly related to the concentration of folate. Growth was measured by spectrophotometry at 610-630 nm. Standards, blanks, and control pools were run with each set of samples. The inter- and intra-assay coefficients of variation for the controls were 10.2% and 7.1%, respectively. The intra-assay coefficient of variation for the subjects' samples was 3.7%. The folate assay was repeated if the optical density of the sample reached or exceeded the highest standard's optical density of approximately 0.95 units with the diluted value from the repeated assay used as the final result. The normal range of serum folate for adult females is 5-25 ng/mL.[55] Deficiency has been defined as serum folate <3 ng/mL for adult females.[56,57] The normal range for serum folate in the first trimester of pregnancy has been reported to be 2.6-15.0 ng/mL.[104] However, deficiency criteria for pregnancy are uncertain.[58] Folate deficiency was defined as serum folate <3 ng/mL.

3.4.2 Determining iron status

Iron status was determined by serum ferritin level analyzed using an immuno-spectrophotometric method with reagents obtained from Beckman-Coulter (Brea, CA). The volume of serum needed for the analysis from each sample was 100 µL. The serum was incubated with a suspension of latex beads coated with a polyclonal rabbit anti-ferritin antibody. After agglutination occurred, the absorbance was measured at 660 nm. Blanks, calibrators, and control pools were run with each set of samples. The inter- and intra-assay coefficients of variation for the controls were 5.0% and 2.7%, respectively. The intra-assay coefficient of variation for the subjects' samples was 3.7%. The assay was repeated for serum ferritin levels of less than 8 ng/mL and for values higher than 300 ng/mL with the initial values used as the final result. The normal range for serum ferritin for adult females is 10-150 ng/mL.[55] Iron deficiency has been defined as serum ferritin level <15 ng/mL for adult females.[79,81] Similarly, for pregnant women, the criteria for diagnosing iron deficiency is serum ferritin <10-15 ng/mL.[105] Iron deficiency was defined as serum ferritin <15 ng/mL.

3.4.3 Time points for analyzing serum samples

To address the second aim of the study, the difference between serum folate and serum ferritin levels were determined for each subject from their baseline (preoperative) serum samples to their one-year postoperative follow-up samples. Serum folate and serum ferritin levels were also used to determine folate and ferritin deficiencies at both time points. The one-year postoperative follow-up visit was selected as the beginning of the 12-18 month recommended waiting period for women to avoid becoming pregnant following bariatric surgery.[11]

3.4.4 Procurement of stored serum samples

The serum samples obtained from the LABS-2 participants were stored at the NIDDK Biospecimen Repository. After receiving approval from the LABS Steering Committee to conduct an ancillary study and use serum samples from participants, a Material Transfer Agreement/Sample and Data Use Agreement was completed between the University of Pittsburgh and the NIDDK Central Repositories. The LABS DCC staff developed a list of serum samples from the randomly selected subjects to be requested from the NIDDK Central Repositories. With two samples from each of the 427 subjects, a total of 854 serum samples were requested. The analysis required a total of 400 µL of serum per sample (300 µL for the folate analysis and 100 µL for the ferritin analysis).

3.5 ADDITIONAL VARIABLES

Data on additional variables of interest were provided by LABS DCC staff. The variables were collected by LABS-trained personnel or participant self-report using standardized instruments and forms.[4,96,100] If data were not reported, they were designated as “missing”.

Type of bariatric surgery, RYGB or LAGB, and age at surgery were reported. Baseline BMI was calculated as weight in kilograms divided by height in meters squared using weight and height obtained at baseline. Pregnancy status (pregnant, potentially pregnant, or not pregnant) at the one-year postoperative visit was self-reported.

Percent weight change is the percentage of the individual’s preoperative body weight that was lost, or gained, between baseline and the one-year follow-up visit.[106] Percent weight change was calculated as weight at baseline minus weight at the one-year postoperative follow-up visit divided by weight at baseline, and multiplied by 100. Thus, a positive value indicates weight loss and a negative value indicates weight gain. Weight data were excluded for any subjects who were pregnant at the time of the one-year postoperative visit.

Race and ethnicity were of interest due to the potential association with folate and iron deficiency. Race was self-reported with participants able to select one or more of the following: White, Black, Asian, American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, or other. Due to the low number of participants identifying as Asian, American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, or of multiple races, these races were combined and categorized as "other or of multiple races". Ethnicity was self-reported as Hispanic or non-Hispanic.

Data on MVI, MVI with minerals, iron, and folate supplement use were used to investigate the hypothesis that micronutrient supplement use is significantly associated with change in serum folate and serum ferritin levels. A MVI is a supplement that contains a combination of different water-soluble and fat-soluble vitamins. MVI use was included as a variable for the folate analyses because MVI supplements commonly include folate. MVI with minerals use is relevant to the serum ferritin analyses because these usually, but not always, contain iron. These data were self-reported by participants at baseline and the one-year postoperative visit.[96] Participants were asked to report if they had taken a MVI or an individual vitamin or mineral supplement (including folate or iron) within the past 90 days, and, if so, to report frequency of use as daily, weekly, monthly/rarely, or no longer taking. Participants who reported taking a MVI were also asked if the MVI included minerals. For the folate analyses, all participants who reported taking a MVI or MVI with minerals at least weekly were classified as taking a MVI. For frequency of supplement use, the categories of monthly/rarely and no longer taking were combined due to the low level of micronutrients that would be provided with infrequent supplement use. When examining the change in serum folate and ferritin, a multiple category variable was created for each type of supplement to compare the use of the supplement on a daily/weekly basis at baseline only, follow-up only, both time points, and neither time point.

The Alcohol Use Disorders Identification Test (AUDIT) was used to assess past-year alcohol use with responses provided by self-report at baseline and the one-year postoperative visit. The AUDIT was developed by the World Health Organization and consists of ten questions (total score of 0-40) used to identify individuals who display harmful consumption patterns of alcohol.[107] A score of 8 or higher indicates harmful alcohol use and possible alcohol dependence with a score of 20 or higher warranting evaluation for alcohol dependence.[107] Thus, participants were classified as positive for alcohol use disorder symptoms if their AUDIT score was $\geq$8. In addition to the AUDIT score, data from individual questions on frequency of alcohol consumption and number of drinks containing alcohol consumed on a typical day were included in the analyses. Only the data from the one-year postoperative visit were used for these alcohol-related variables.

Frequency of breakfast consumption was investigated for association with change in serum folate since foods commonly consumed at breakfast such as fortified cereals and orange juice are good dietary sources of folate and folic acid.[50] Participants reported the number of days in a usual week that breakfast was consumed at baseline and at one-year follow-up. Only the data from baseline were examined for the study. One-year postoperative breakfast consumption would be less relevant due to the small size of meals and specific diet recommendations for individuals after bariatric surgery. For the statistical analyses, frequency of breakfast consumption reported at baseline was categorized as: 0-2 times per week, 3-5 times per week, or 6-7 times per week.

Additional factors that may be associated with folate or iron deficiency are income level and education level. Individuals of lower socioeconomic status are at risk for deficiencies of folate and iron due to poor quality diets with decreased intake of micronutrient-rich foods.[82,108] NHANES data have shown a significant association ($p<.001$) between poverty and lower serum folate levels.[61] Annual household income was self-reported in LABS-2 at baseline with the following categories: <$25,000, $25,000 - $49,999, $50,000 - $74,999, $75,000-$99,999, or ≥$100,000. Highest education level completed was self-reported at baseline with participants selecting from eight categories (less than seventh grade, seventh to less than ninth grade, some high school, high school diploma or General Equivalency Degree (GED), some college, other post-high school education, college diploma, or graduate or professional degree). For these analyses, the household income categories were condensed to the following: <$25,000, $25,000 - $49,999, $50,000 - $74,999, and ≥$75,000, and the education categories were condensed to: high school diploma or GED or less, some college or post-high school education, and college degree or greater.

Participants self-reported the name and frequency of use (daily, weekly, monthly/rarely, as needed, or no longer taking) for any prescription medications taken within the past 90 days. The list of prescription medications reported by each participant at the one-year postoperative visit was reviewed to identify specific medications known to be associated with deficiencies of folate or iron. For example, histamine H_2 receptor antagonists can decrease absorption of iron.[109]

3.6 STATISTICAL ANALYSIS

SAS version 9.4 (SAS Institute) and Stata version 14.2 (StataCorp) were used to conduct the statistical analyses. *P* values of <.05 were considered statistically significant for all analyses. Descriptive statistics were calculated for all variables. For continuous variables, the mean (for normally distributed variables) or the median, 25th and 75th percentiles, standard deviation, and range were reported. Frequencies and percentages were reported for categorical variables. Normality of variables was assessed using histograms, Q-Q plots, and the Shapiro-Wilk test. Distributions of continuous variables were assessed visually for skewness and outliers. Two-sample t-tests or analysis of variance (ANOVA) were used to test for differences in means between groups for normally distributed continuous variables. The Mann-Whitney U test or Kruskal-Wallis test were used to test differences between, or among, groups for continuous variables when not normally distributed. For categorical variables, the Pearson's chi-square test or Fisher's exact test were used to test for significant differences between, or among, groups.

To address the second aim of the study, the change in serum folate and change in serum ferritin level from baseline to one year following bariatric surgery were determined for each subject. Scatter plots and box plots were used to visually assess the relationship between individual variables and the change in serum folate or ferritin. Multivariable linear regression was used to assess the relationship between type of surgery and mean change in serum folate and serum ferritin from baseline to one year following surgery, adjusting for covariates. To account for possible site differences, LABS clinical sites were included as fixed effects. The LABS clinical site with the largest percentage of subjects (32.4%) was used as the reference category of site. The covariates included in the change in serum folate model were: age at surgery, race, ethnicity, annual household income, highest level of education completed, baseline BMI, percent weight change from baseline, MVI supplement use on a daily/weekly basis (at neither baseline or one-year follow-up, at baseline only, at follow-up only, at both baseline and follow-up), folate supplement use on a daily/weekly basis (at neither baseline or one-year follow-up, at baseline only, at follow-up only, at both baseline and follow-up), baseline serum folate level, alcohol use disorder symptoms at one-year follow-up (as determined by AUDIT score), frequency of alcohol consumption reported at one-year follow-up, number of alcoholic beverages consumed on a typical day reported at one-year follow-up, and frequency of breakfast consumption reported at baseline. Age at surgery and baseline BMI were centered at their median values. The same variables were adjusted for in the change in serum ferritin model with the following exceptions: MVI with minerals supplement use on a daily/weekly basis (at neither baseline or one-year follow-up, at baseline only, at follow-up only, at both baseline and follow-up) replaced MVI supplement use, iron supplement use on a daily/weekly basis (at neither baseline or one-year follow-up, at baseline only, at follow-up only, at both baseline and follow-up) replaced folate supplement use, and baseline serum ferritin level replaced baseline serum folate level. Use of histamine H_2 receptor antagonist medication reported at the one-year postoperative visit was considered in the change in serum ferritin model, and the alcohol use variables at one-year postoperatively and frequency of breakfast consumption at baseline were not.

The prevalence of deficiency of each micronutrient at both baseline and one year after surgery was determined for both types of surgery. Box plots were used to visually assess the relationship between each continuous variable and the presence or absence of deficiency. Contingency tables with frequencies and prevalence percentages were created for categorical variables. Pearson's chi-square test or Fisher's exact test were used to test if the prevalence of deficiency differed significantly between, or among, groups. The Mantel-Haenszel chi-square test was used to test for significant linear trends for ordinal variables. A test of symmetry was conducted to assess change in the distributions of folate and ferritin sufficiency status (deficient, within the normal range, or above the normal range/high) from baseline to one year after surgery. Multivariable logistic regression was used to assess the relationship between type of surgery and presence of ferritin deficiency at one year following surgery, while adjusting for the following covariates: age at surgery, race, ethnicity, annual household income, highest level of education completed, baseline BMI, ferritin deficiency status at baseline, percent weight change from baseline, MVI including minerals supplement use one-year postoperatively, iron supplement use one-year postoperatively, and use of histamine H_2 receptor antagonist medication one-year postoperatively. As will be discussed in the "Results" section, multivariable logistic regression was not conducted for folate deficiency due to a lack of subjects with deficiency at one-year follow-up.

To address the third aim of the study, multivariable linear regression was used to assess the association of baseline and post-operative factors with change in serum folate and serum ferritin among women who underwent RYGB. LABS clinical site was included in the model as fixed effects. The same covariates as listed above were examined for association with change serum folate and serum ferritin.

The following process was used for multivariable linear and logistic models. Simple regression models were created to assess the linear relationship between each variable and the outcome. An initial multivariable base model was created that included the demographic variables, BMI at baseline, percent weight change from baseline, supplement use variables, and LABS clinical site as fixed effects. Next, a parsimonious model was created which included significant variables from the base model, significant variables from the simple regression models using the multiple degree of freedom test for categorical variables, and LABS clinical site as fixed effects. Using stepwise regression, all other variables were then added to the parsimonious model stepwise with those variables with $p<.05$ remaining in the model with the exception of race and supplement use variables which were maintained in the models regardless of statistical significance. Race and supplement use are of particular interest due to potential confounding by these variables, and the study may have been underpowered to detect a significant association due to low prevalence (e.g. African American race) or missing data (e.g. supplement use). For multiple category variables (i.e. supplement use, annual household income), all categories of a variable were added stepwise at the same time. All two-way interactions were tested for variables in each final main effects model. Parameter estimates for multivariable linear regression models were determined. For multivariable logistic regression models, point estimates, adjusted odds ratios, and 95% confidence intervals were determined. Assumptions were tested for each final model.

4.0 RESULTS

4.1 SUBJECTS

The study was designed to have 427 subjects with 280 subjects (66%) in the RYGB group and 147 subjects (34%) in the LAGB group (Figure 6). However, the final sample was 426 because the serum samples for one LAGB subject were hemolyzed and could not be used for analyses so this subject was excluded. Replacement of this subject was not an option because there were no other subjects who had undergone LAGB who met the inclusion criteria for the study.

Demographic and other select characteristics for the subjects are presented in Table 8. The median age was 37 years in both surgery groups. Most subjects, 86.5%, were white while 10.4% were African American, and 3.1% of subjects reported being of other or multiple races. Only, 7.7% of the subjects were Hispanic. There were no significant differences in the distribution of race or ethnicity by surgery. The distribution of education differed significantly by surgical procedure (p=.002). A larger percentage of subjects having a LAGB, 50.7%, compared to those undergoing a RYGB, 32.7%, reported at least a college education. The distribution of household income also differed significantly by surgery (p=.0007). A larger percentage of subjects having a LAGB, 39.5%, compared to those undergoing a RYGB, 22.2%, reported an annual household income of $75,000 or more. Baseline BMI was significantly higher (p=.0003) for subjects who underwent a RYGB (median 46.6 kg/m^2) compared to LAGB (median 44.3 kg/m^2). The median percent weight change was significantly greater ($p<.0001$) for subjects who had undergone RYGB (median 36.0%) compared to LAGB (median 14.2%).

At baseline, 50.6% of subjects reported taking a MVI daily, 47.2% reported taking a MVI that included minerals daily, 10.9% reported taking an iron supplement either daily or weekly, and 2.0% of subjects reported taking a folate supplement daily or weekly. These percentages increased substantially one year after surgery when 75.3% of the subjects reported taking a MVI daily, 71.5% reported taking a MVI that included minerals daily, 34.9% reported taking an iron supplement either daily or weekly, and 7.7% of subjects reported taking a folate supplement daily or weekly. At baseline, there were no significant differences in distributions of MVI, MVI with minerals, iron, or folate supplement use by type of surgery. The percentage of subjects who took an MVI, MVI including minerals, iron, and folate supplement use at one-year follow-up differed by surgery ($p<.0001$, $p=.004$, $p<.0001$, and $p=.02$, respectively). Subjects who had undergone RYGB compared to LAGB were more likely to take a MVI daily (83.8% vs. 58.0%), MVI including minerals daily (78.9% vs. 56.5%), iron supplement daily or weekly (46.5% vs. 11.1%), or a folate supplement daily or weekly (10.0% vs. 3.1%).

Prior to surgery, 53.7% of subjects reported consuming breakfast six to seven times per week, while 13.1% reported consuming breakfast two or less times per week. The distribution of frequency of breakfast consumption did not differ by surgery. At baseline, a larger percentage of subjects who had a LAGB, 5.8%, reported using a histamine H2 receptor antagonist medication compared to 1.9% of subjects who underwent RYGB ($p=.04$). One year after surgery, the use of a histamine H2 receptor antagonist medication did not differ significantly by surgery

Prior to surgery, 36.8% of subjects reported never drinking alcohol and only three subjects (0.7%) reported consuming alcohol four or more times per week. These percentages were similar after surgery as 37.9% of subjects reported never drinking alcohol and 1.9% of subjects reported consuming alcohol four or more times per week. Frequency of alcohol consumption did not differ significantly by surgical procedure at either baseline or one year after surgery. Before surgery, 22.5% of subjects reported drinking three or more alcoholic beverages per day on a typical day when drinking. One year after surgery, this percentage decreased to 18.5%. A larger percentage of subjects who underwent RYGB, 24.4%, reported consuming three or more alcoholic drinks per day on a typical day when drinking prior to surgery compared to 18.9% of subjects who underwent LAGB. Whereas, one year after surgery, a larger percentage of subjects who had undergone LAGB compared to RYGB reported consuming three or more alcoholic drinks per day on a typical day when drinking (23.4% vs. 16.1%, respectively). For the AUDIT score, 5.4% and 5.9% of subjects received a score indicating harmful alcohol use and possible alcohol dependence at baseline and one year after surgery, respectively. The AUDIT score did not differ significantly by surgery at either time point.

At the one-year postoperative visit, four subjects reported being pregnant. Nine subjects reported possibly being pregnant. Pregnancy status did not differ significantly by surgery.

Table 8: Demographic and select characteristics of subjects

		Total (n=426)	LAGB (n=146)	RYGB (n=280)	p
Age (years)	Median	37	37	37	0.39
	25^{th}, 75^{th} %ile	31, 41	32, 42	31, 41	
	Range	18-45	18-45	19-45	
	Missing (n, %)	0 (0)	0 (0)	0 (0)	
		n (%)	n (%)	n (%)	
Race					0.28
White		365 (86.5)	128 (89.5)	237 (85.0)	
Black		44 (10.4)	13 (9.1)	31 (11.1)	
Other or multiple races		13 (3.1)	2 (1.4)	11 (3.9)	
Missing		4 (0.9)	3 (2.1)	1 (0.4)	
Ethnicity					0.52
Hispanic		33 (7.7)	13 (8.9)	20 (7.1)	
Non-Hispanic		393 (92.3)	133 (91.1)	260 (92.9)	
Missing		0 (0)	0 (0)	0 (0)	
Education					0.002
≤ High school diploma		84 (20.8)	23 (16.7)	61 (22.9)	
Some college or post-high school		163 (40.3)	45 (32.6)	118 (44.4)	
≥ College degree		157 (38.9)	70 (50.7)	87 (32.7)	
Missing		22 (5.2)	8 (5.5)	14 (5.0)	
Household income					0.0007
<$25,000		75 (19.0)	19 (14.2)	56 (21.5)	
$25,000-$49,999		109 (27.6)	26 (19.4)	83 (31.8)	
$50,000-$74,999		100 (25.3)	36 (26.9)	64 (24.5)	
≥ $75,000		111 (28.1)	53 (39.5)	58 (22.2)	
Missing		31 (7.3)	12 (8.2)	19 (6.8)	

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; %ile: percentile

Table 8 (continued): Demographic and select characteristics of subjects

		Total (n=426)	LAGB (n=146)	RYGB (n=280)	p
Baseline BMI (kg/m²)[a]	Median	45.9	44.3	46.6	0.0003
	25th, 75th %ile	42.0, 50.8	41.0, 48.4	42.8, 51.6	
	Range	32.9-74.7	32.9-66.4	35.4-74.7	
	Missing (n, %)	0 (0)	0 (0)	0 (0)	
Percent weight change from baseline at 1-year post-op (%)[b]	Median	31.4	14.2	36.0	<.0001
	25th, 75th %ile	17.9, 37.9	10.2, 19.9	30.7, 40.9	
	Range	58.5% loss to 8.6% gain	53.0% loss to 1.4% gain	58.4% low to 8.6% gain	
	Missing (n, %)[c]	4 (0.9)	1 (0.7)	3 (1.1)	
		n (%)	n (%)	n (%)	
MVI use, baseline					0.41
Daily		203 (50.6)	65 (47.8)	138 (52.1)	
Weekly		37 (9.2)	16 (11.8)	21 (7.9)	
Monthly/rarely or no current use		161 (40.2)	55 (40.4)	106 (40.0)	
Missing		25 (5.9)	10 (6.8)	15 (5.4)	
MVI use, 1 year post-op					<.0001
Daily		290 (75.3)	73 (58.0)	217 (83.8)	
Weekly		52 (13.5)	27 (21.4)	25 (9.6)	
Monthly/rarely or no current use		43 (11.2)	26 (20.6)	17 (6.6)	
Missing		41 (9.6)	20 (13.7)	21 (7.5)	
MVI including minerals use, baseline					0.33
Daily		180 (47.2)	60 (45.8)	120 (48.0)	
Weekly		35 (9.2)	16 (12.2)	19 (7.6)	
Monthly/rarely or no current use		166 (43.6)	55 (42.0)	111 (44.4)	
Missing		45 (10.6)	15 (10.3)	30 (10.7)	
MVI including minerals use, 1 year post-op					0.004
Daily		263 (71.5)	69 (56.5)	194 (78.9)	
Weekly		49 (13.3)	25 (20.5)	24 (9.7)	
Monthly/rarely or no current use		56 (15.2)	28 (23.0)	28 (11.4)	
Missing		58 (13.6)	24 (16.4)	34 (12.1)	

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; %ile: percentile; BMI: body mass index; post-op: postoperative; MVI: multivitamin

[a]Calculated as weight in kilograms divided by height in meters squared.

[b]Calculated as 100*(weight at baseline minus weight at 1 year post-op divided by weight at baseline).

[c]Weight data were excluded for three subjects in their second or third trimester of pregnancy and one subject who was less than six months postpartum.

Table 8 (continued): Demographic and select characteristics of subjects

	Total (n=426)	LAGB (n=146)	RYGB (n=280)	p
	n (%)	n (%)	n (%)	
Iron supplement use, baseline				0.17
Daily or weekly	44 (10.9)	11 (8.0)	33 (12.4)	
Monthly/rarely or no current use	359 (89.1)	127 (92.0)	232 (87.6)	
Missing	23 (5.4)	8 (5.5)	15 (5.4)	
Iron supplement use, 1 year post-op				<.0001
Daily or weekly	134 (34.9)	14 (11.1)	120 (46.5)	
Monthly/rarely or no current use	250 (65.1)	112 (88.9)	138 (53.5)	
Missing	42 (9.9)	20 (13.7)	22 (7.9)	
Folate supplement use, baseline				0.72
Daily or weekly	8 (2.0)	2 (1.4)	6 (2.3)	
Monthly/rarely or no current use	396 (98.0)	136 (98.6)	260 (97.7)	
Missing	22 (5.2)	8 (5.5)	14 (5.0)	
Folate supplement use, 1 year post-op				0.02
Daily or weekly	30 (7.7)	4 (3.1)	26 (10.0)	
Monthly/rarely or no current use	359 (92.3)	124 (96.9)	235 (90.0)	
Missing	37 (8.7)	18 (12.3)	19 (6.8)	
Breakfast consumption, prior to surgery				0.83
0-2 times/week	53 (13.1)	17 (12.3)	36 (13.5)	
3-5 times/week	134 (33.2)	44 (31.9)	90 (33.8)	
6-7 times/week	217 (53.7)	77 (55.8)	140 (52.6)	
Missing	22 (5.2)	8 (5.5)	14 (5.0)	
Use of a histamine H2 receptor antagonist medication, baseline				0.04
Yes	13 (3.2)	8 (5.8)	5 (1.9)	
No	389 (96.8)	129 (94.2)	260 (98.1)	
Missing	24 (5.6)	9 (6.2)	15 (5.4)	
Use of a histamine H2 receptor antagonist medication, 1 year post-op				0.21
Yes	27 (7.0)	6 (4.7)	21 (8.1)	
No	359 (93.0)	122 (95.3)	237 (91.9)	
Missing	40 (9.4)	18 (12.3)	22 (7.9)	

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; post-op: postoperative

Table 8 (continued): Demographic and select characteristics of subjects

	Total (n=426)	LAGB (n=146)	RYGB (n=280)	p
	n (%)	n (%)	n (%)	
Frequency of alcohol consumption, baseline				0.43
Never	149 (36.8)	46 (33.1)	103 (38.7)	
<u><</u>2-4 times/month	232 (57.3)	84 (60.4)	148 (55.6)	
2-3 times/week	21 (5.2)	7 (5.0)	14 (5.3)	
<u>></u>4 times/week	3 (0.7)	2 (1.5)	1 (0.4)	
Missing	21 (4.9)	7 (4.8)	14 (5.0)	
Frequency of alcohol consumption, 1 year post-op				0.13
Never	142 (37.9)	37 (29.8)	105 (41.8)	
<u><</u>2-4 times/month	208 (55.4)	77 (62.1)	131 (52.2)	
2-3 times/week	18 (4.8)	7 (5.7)	11 (4.4)	
<u>></u>4 times/week	7 (1.9)	3 (2.4)	4 (1.6)	
Missing	51 (12.0)	22 (15.1)	29 (10.4)	
Number of alcoholic drinks consumed on a typical day of drinking, baseline				0.04
None/not applicable	150 (37.1)	46 (33.3)	104 (39.1)	
1-2 drinks/day	163 (40.4)	66 (47.8)	97 (36.5)	
3-6 drinks/day	83 (20.5)	26 (18.9)	57 (21.4)	
<u>></u> 7 drinks/day	8 (2.0)	0 (0)	8 (3.0)	
Missing	22 (5.2)	8 (5.5)	14 (5.0)	
Number of alcoholic drinks consumed on a typical day of drinking, 1 year post-op				0.07
None/not applicable	142 (38.2)	39 (31.4)	103 (41.5)	
1-2 drinks/day	161 (43.3)	56 (45.2)	105 (42.3)	
3-6 drinks/day	66 (17.7)	29 (23.4)	37 (14.9)	
<u>></u> 7 drinks/day	3 (0.8)	0 (0)	3 (1.2)	
Missing	54 (12.7)	22 (15.1)	32 (11.4)	

<u>Abbreviations</u>: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; post-op: postoperative

Table 8 (continued): Demographic and select characteristics of subjects

	Total (n=426)	LAGB (n=146)	RYGB (n=280)	p
	n (%)	n (%)	n (%)	
Alcohol use disorder symptoms, baseline[d]				0.47
Yes	22 (5.4)	6 (4.3)	16 (6.0)	
No	383 (94.6)	133 (95.7)	250 (94.0)	
Missing	21 (4.9)	7 (4.8)	14 (5.0)	
Alcohol use disorder symptoms, 1 year post-op[d]				0.55
Yes	22 (5.9)	6 (4.8)	16 (6.4)	
No	352 (94.1)	118 (95.2)	234 (93.6)	
Missing	52 (12.2)	22 (15.1)	30 (10.7)	
Pregnancy status, 1 year post-op				1.00
Pregnant	4 (0.9)	1 (0.7)	3 (1.1)	
Possibly pregnant	9 (2.1)	3 (2.0)	6 (2.1)	
Not pregnant	413 (97.0)	142 (97.3)	271 (96.8)	
Missing	0 (0)	0 (0)	0 (0)	

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; post-op: postoperative

[d]Based on score on the Alcohol Use Disorders Identification Test (AUDIT). An AUDIT score $\geq$ 8 indicates harmful alcohol use and possible alcohol dependence.

4.2 FOLATE

4.2.1 Serum folate levels at baseline and follow-up and change in serum folate

The Heinz Nutrition Laboratory was unable to determine either a preoperative or postoperative serum folate level for 13 subjects, so the sample size for the folate analyses was 413 (272 RYGB, 141 LAGB). Table 9 presents descriptive statistics for baseline serum folate by type of surgery. The median values at baseline for subjects who underwent LAGB and RYGB were 27.2 ng/mL (range: 5.2-67.9 ng/mL) and 23.0 ng/mL (range: 2.4-65.9 ng/mL), respectively. The normal range for serum folate for adult females is 5-25 ng/mL,[55] and deficiency has been defined as serum folate <3 ng/mL for adult females.[56,57] For this analysis, a serum folate level of <3 ng/mL was considered below the normal range (deficient), a serum folate in the range of 3-25 ng/mL was considered within the normal range, and a serum folate level >25 ng/mL was considered above the normal range (high).

Table 9: Serum folate at baseline

	Mean (ng/mL)	SD	25th %ile (ng/mL)	Median (ng/mL)	75th %ile (ng/mL)	Range (ng/mL)	Below normal range: n (%)	Within normal range: n (%)	Above normal range: n (%)
LAGB (n=141)	27.2	11.6	18.4	26.4	36.2	5.2-67.9	0 (0%)	64 (45.4%)	77 (54.6%)
RYGB (n=272)	23.0	12.2	12.0	22.2	31.1	2.4-65.9	1 (0.4%)	159 (58.4%)	112 (41.2%)

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; SD: standard deviation; %ile: percentile

Table 10 presents descriptive statistics for serum folate at the one-year postoperative visit by surgery. Median values for subjects who underwent LAGB and RYGB were 22.3 ng/mL (range: 2.4-57.8 ng/mL) and 26.0 ng/mL (range: 1.25-74.0 ng/mL), respectively.

Table 10: Serum folate at the one-year postoperative visit

	Median (ng/mL)	25th %ile (ng/mL)	75th %ile (ng/mL)	Range (ng/mL)	Below normal range: n (%)	Within normal range: n (%)	Above normal range: n (%)
LAGB (n=141)	22.3	10.9	34.3	2.4-57.8	1 (0.7%)	78 (55.3%)	62 (44.0%)
RYGB (n=272)	26.0	12.1	37.0	1.25-74.0	3 (1.1%)	125 (46.0%)	144 (52.9%)

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; %ile: percentile

The change in serum folate from baseline to one year following surgery was determined for all subjects by subtracting the baseline serum level from the one-year postoperative visit serum level. A negative value indicates a decrease in serum folate from baseline to the one-year post-operative visit. Descriptive statistics are presented in Table 11. Mean change for subjects who underwent LAGB was a decrease of 4.3 ng/mL with a range from a decrease of 45.7 ng/mL to an increase of 40.1 ng/mL. For subjects who underwent RYGB, mean change was an increase of 2.8 ng/mL with a range from a decrease of 34.3 ng/ml to an increase of 47.9 ng/mL.

Table 11: Change in serum folate level from baseline to the one-year postoperative visit

	Mean (ng/mL)	SD	25th %ile (ng/mL)	Median (ng/mL)	75th %ile (ng/mL)	Range (ng/mL)
LAGB (n=141)	-4.3	14.8	-13.4	-4.4	5.7	-45.7 to 40.1
RYGB (n=272)	2.8	15.9	-6.8	1.5	11.4	-34.3 to 47.9

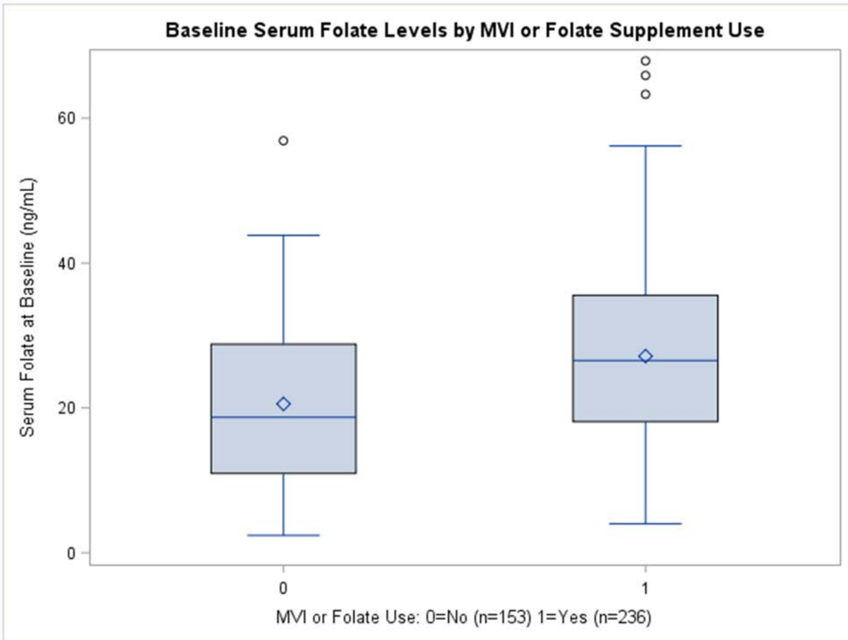
Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; SD: standard deviation; %ile: percentile

4.2.1.1 Investigation of high serum folate levels

Due to the large percentage of subjects with serum folate levels above the normal range at both baseline and one year following bariatric surgery, analyses were conducted to determine if an association existed between serum levels and MVI or folate supplement use or fasting status at the time of blood draw.

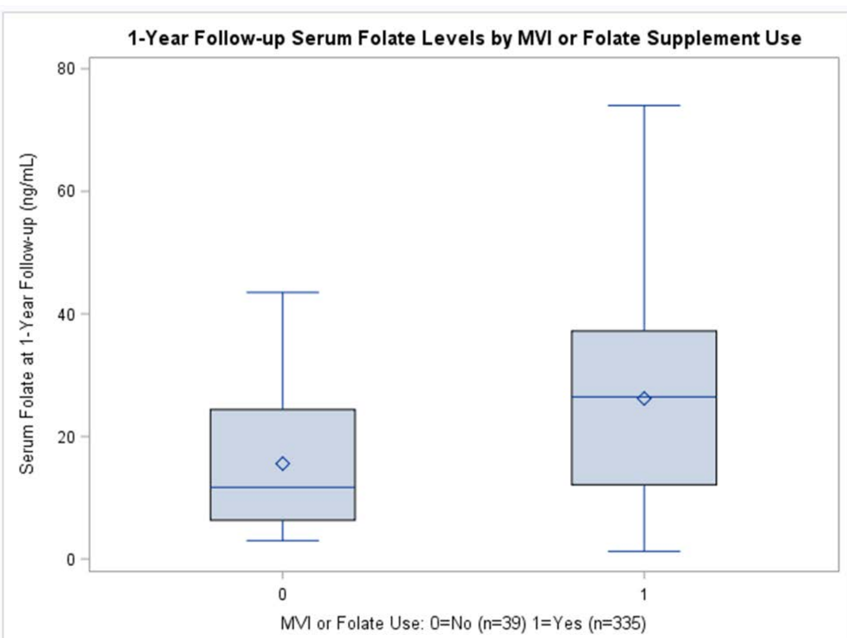
Serum folate levels and MVI or folate supplement use

MVI supplements commonly contain folic acid. For the purpose of these analyses, “MVI or folate supplement use” was defined as daily or weekly reported use of MVI and/or folate supplements. Reported frequency of use of less than weekly was classified as no MVI or folate supplement use. Box plots of serum folate levels and reported MVI or folate supplement use at baseline and the one-year postoperative visit are presented in Figure 7 and Figure 8. The box plots at both time points indicated higher serum folate levels for those subjects who reported taking an MVI and/or folate supplement on a daily or weekly basis. The differences in baseline mean serum folate levels and one-year follow-up median serum folate levels based on MVI or folate supplement use were significant ($p<.0001$ for both time points).



Abbreviations: MVI: multivitamin

Figure 7: Box plot to assess baseline serum folate levels by MVI and/or folate supplement use reported at baseline



Abbreviations: MVI: multivitamin

Figure 8: Box plot to assess one-year postoperative serum folate levels by MVI and/or folate supplement use reported at the one-year postoperative visit

Serum folate levels and fasting status

Fasting is not required for serum folate testing; however, an eight-hour fast is often recommended.[55,57] The inclusion criteria did not require fasting serum samples. The fasting status of each participant was recorded by LABS personnel for both the baseline and one-year postoperative visit blood draws. "Fasting" was defined as fasting for eight or more hours prior to the blood draw and "non-fasting" was defined as no fasting or fasting for less than eight hours. Box plots and statistical analyses were conducted to determine if an association existed between fasting status and serum folate levels.

For the baseline samples, 85.7% of the samples from which serum folate levels were determined were from fasting samples. For the one-year postoperative serum folate levels, 78.2% were obtained from fasting samples. The box plots (Figure 9 and Figure 10) and statistical testing did not suggest an association between fasting status and serum folate levels at baseline ($p=.07$) or the one-year postoperative visit ($p=.55$).

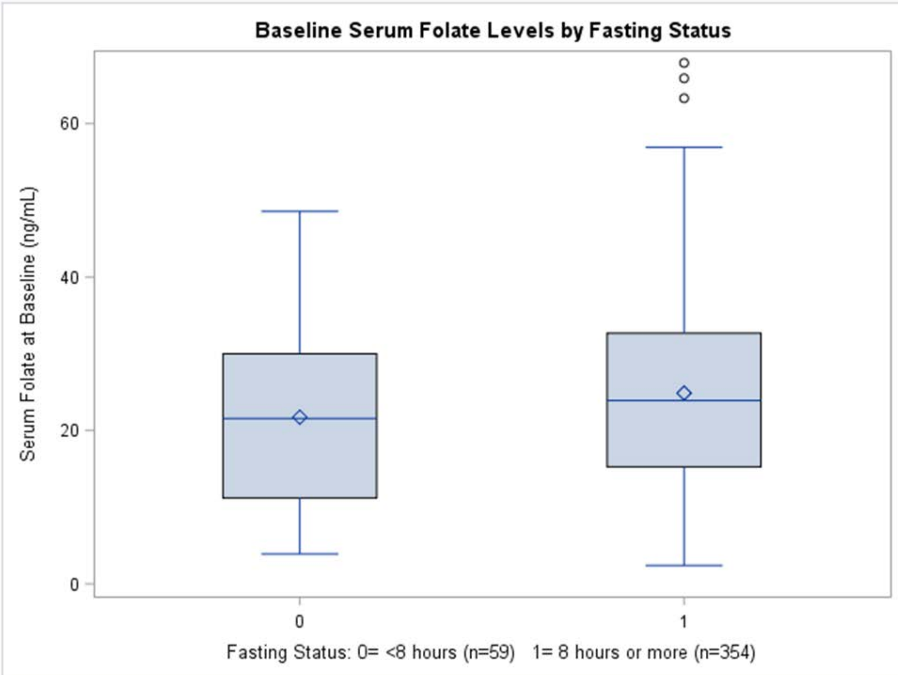


Figure 9: Box plot to assess serum folate levels at baseline by fasting status

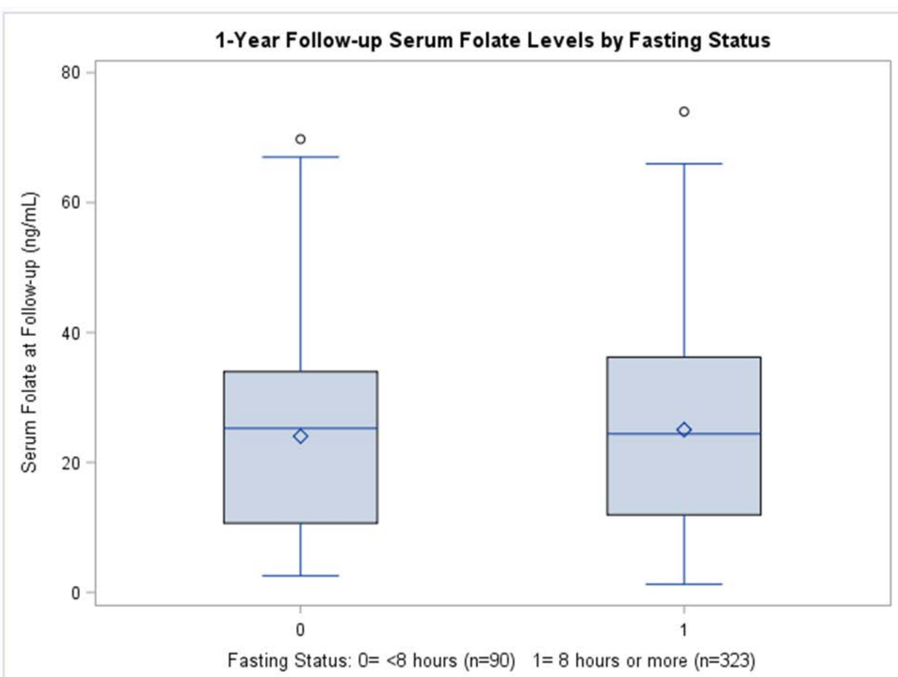


Figure 10: Box plot to assess serum folate levels at one-year postoperative visit by fasting status

4.2.2 Association of bariatric surgery and change in serum folate

Plots of individual covariates versus change in serum folate are presented in Figure 11 and Figure 12.

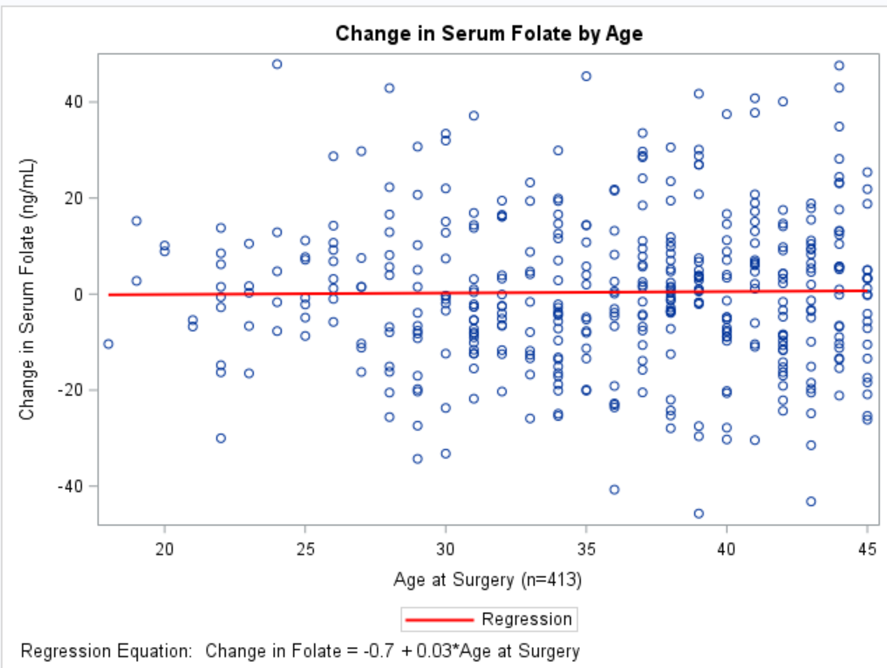
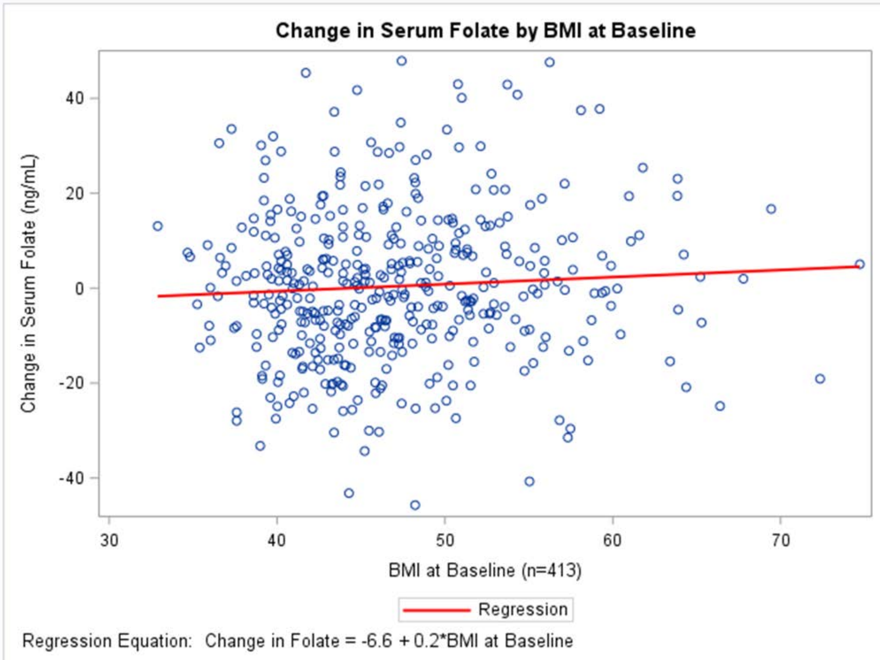


Figure 11: Scatter plots of continuous variables and change in serum folate



Abbreviations: BMI: body mass index

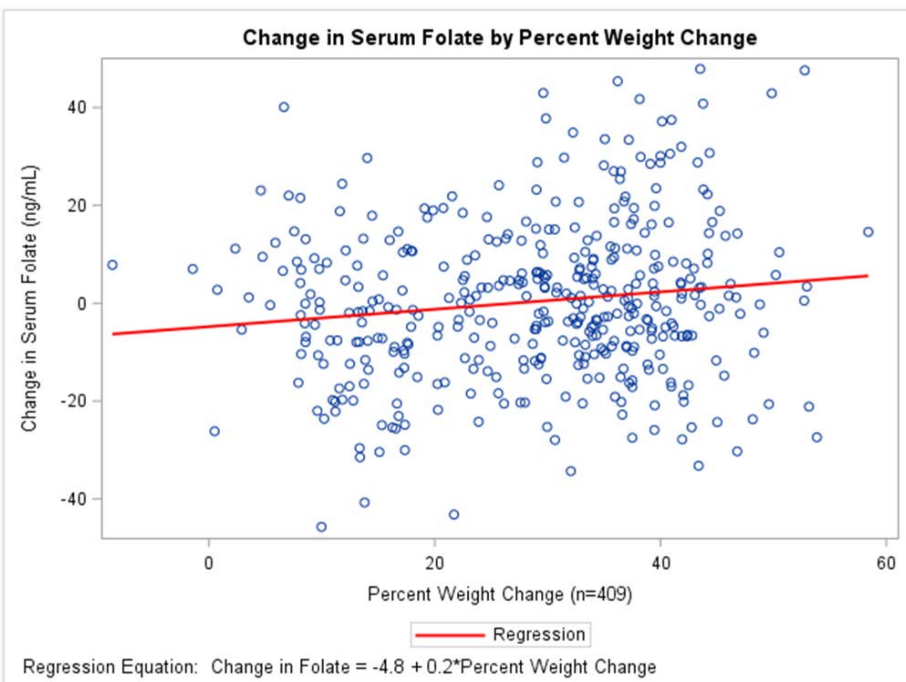
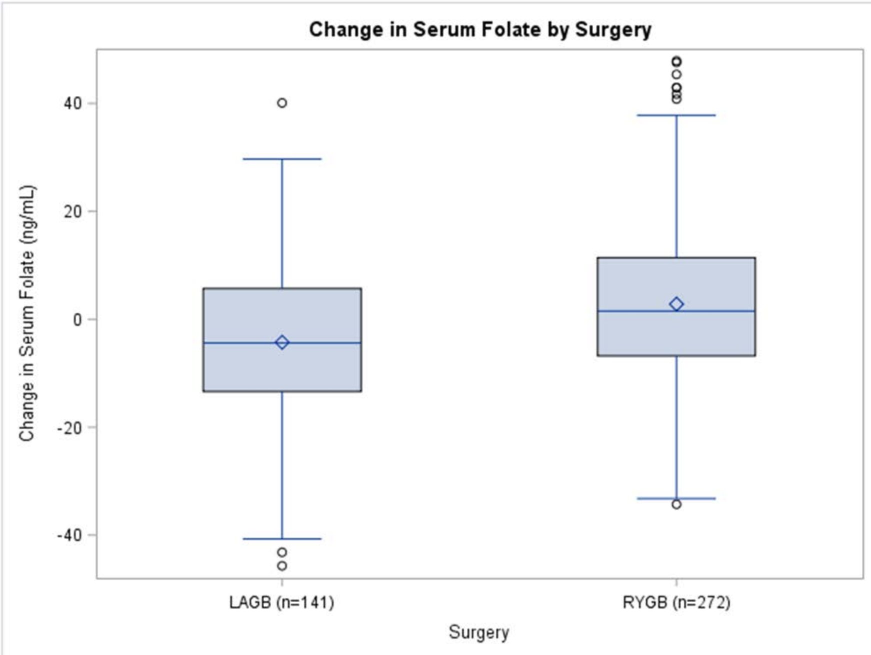


Figure 11 (continued): Scatter plots of continuous variables and change in serum folate



Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass

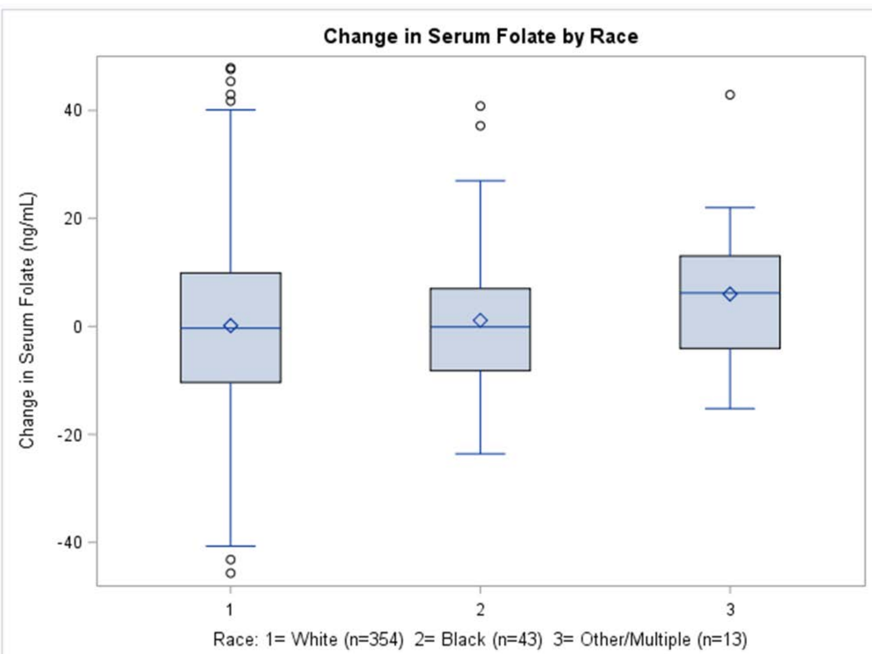


Figure 12: Box plots of categorical variables and change in serum folate

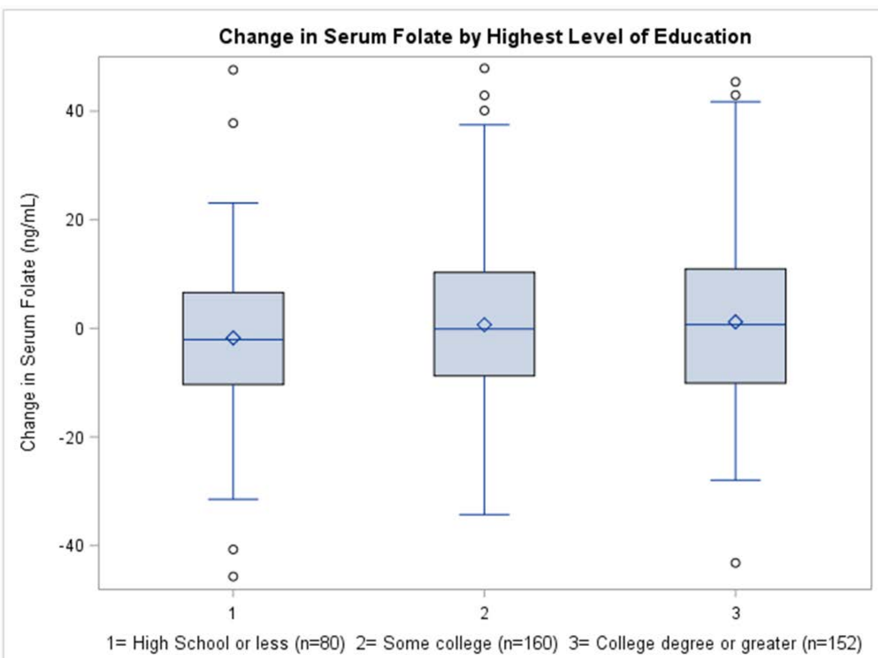
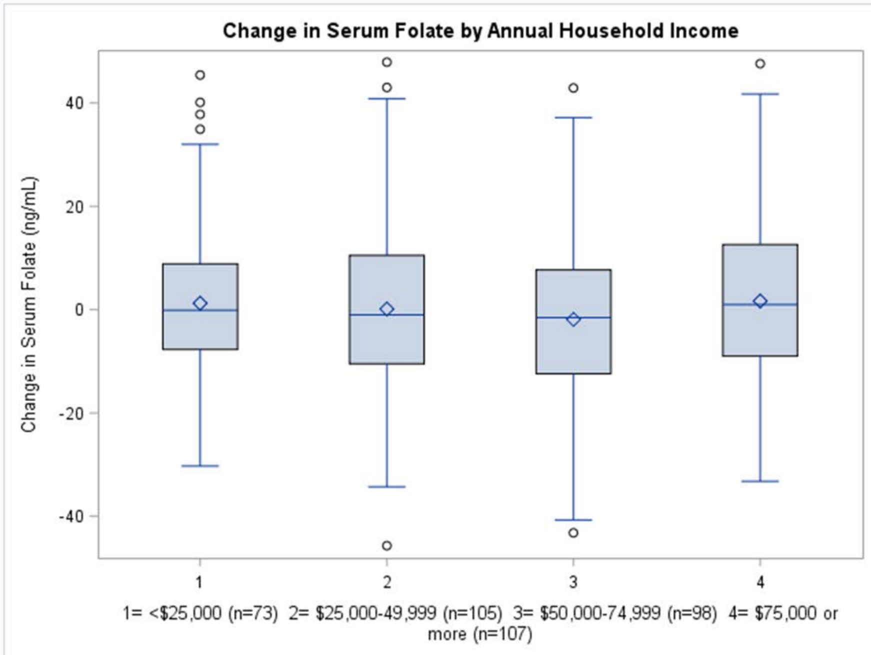
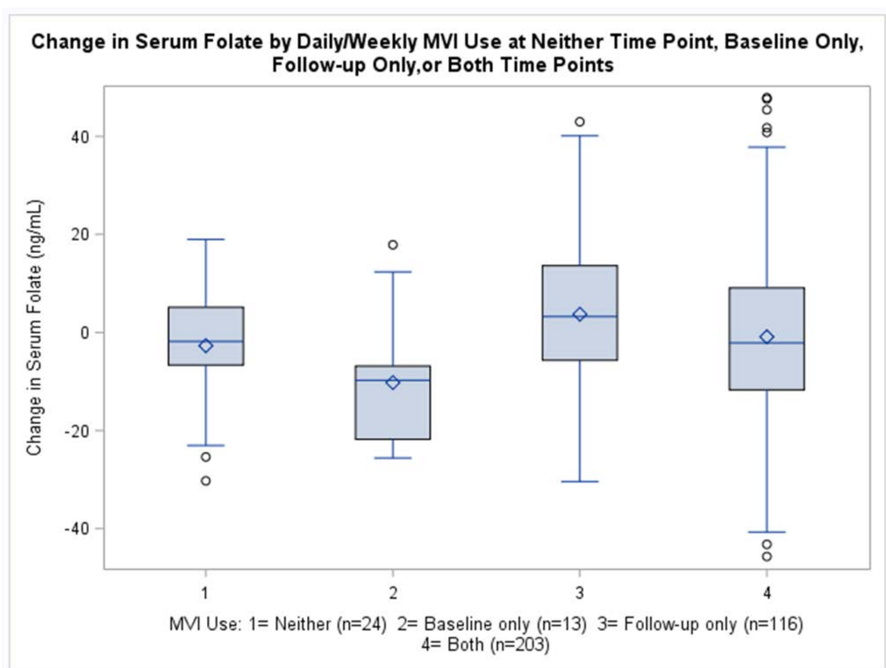


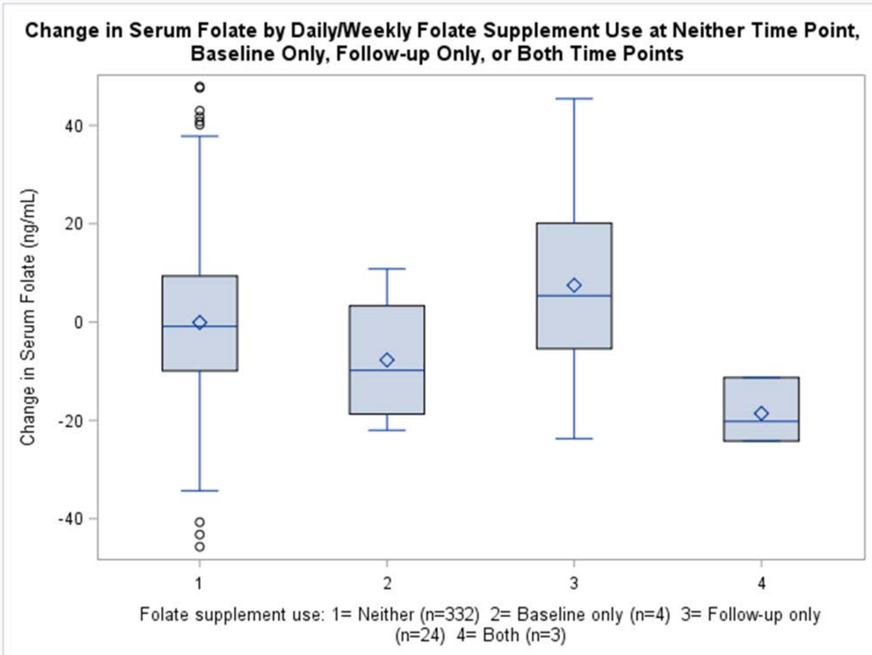
Figure 12 (continued): Box plots of categorical variables and change in serum folate





Abbreviations: MVI: multivitamin

Figure 12 (continued): Box plots of categorical variables and change in serum folate



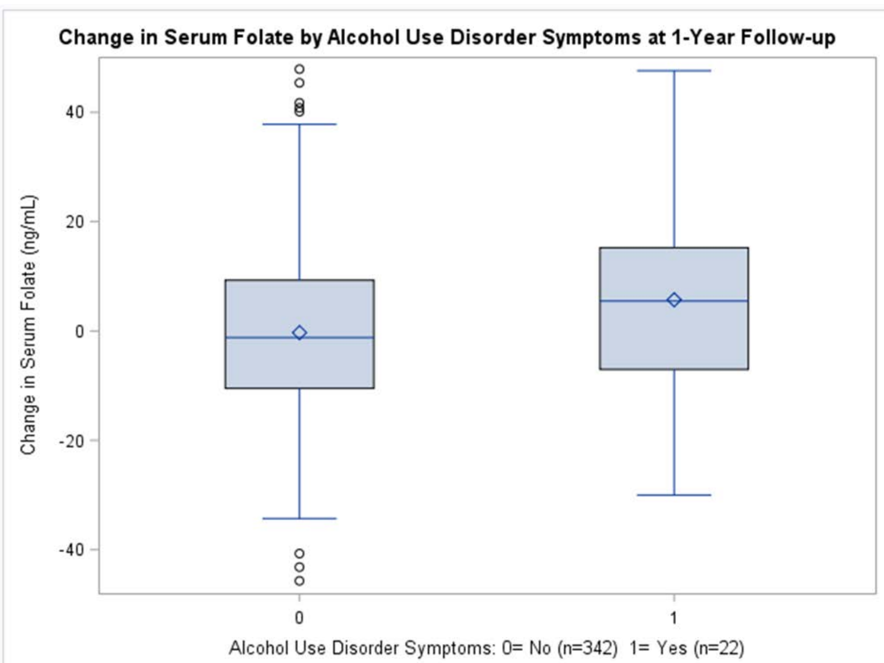
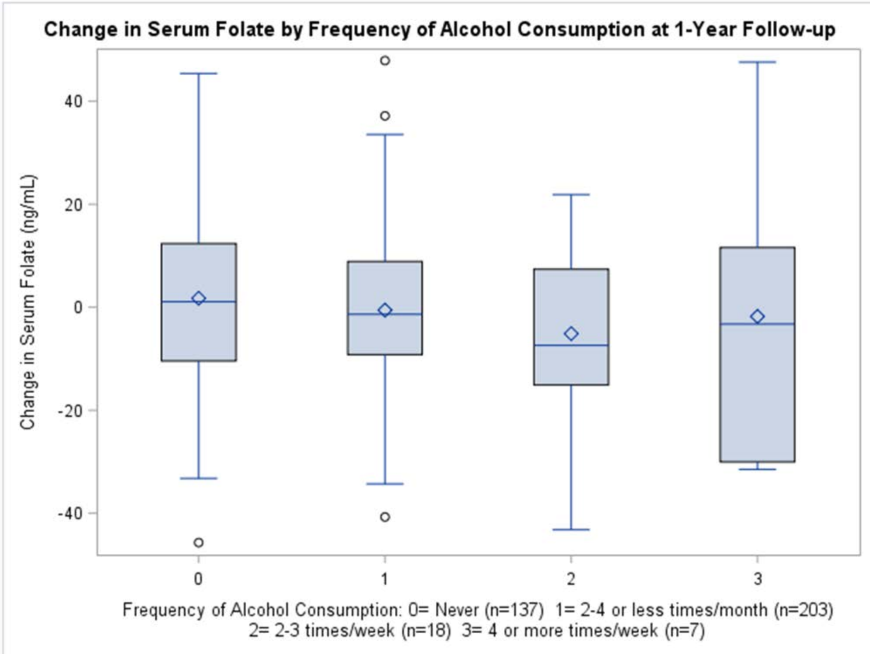


Figure 12 (continued): Box plots of categorical variables and change in serum folate



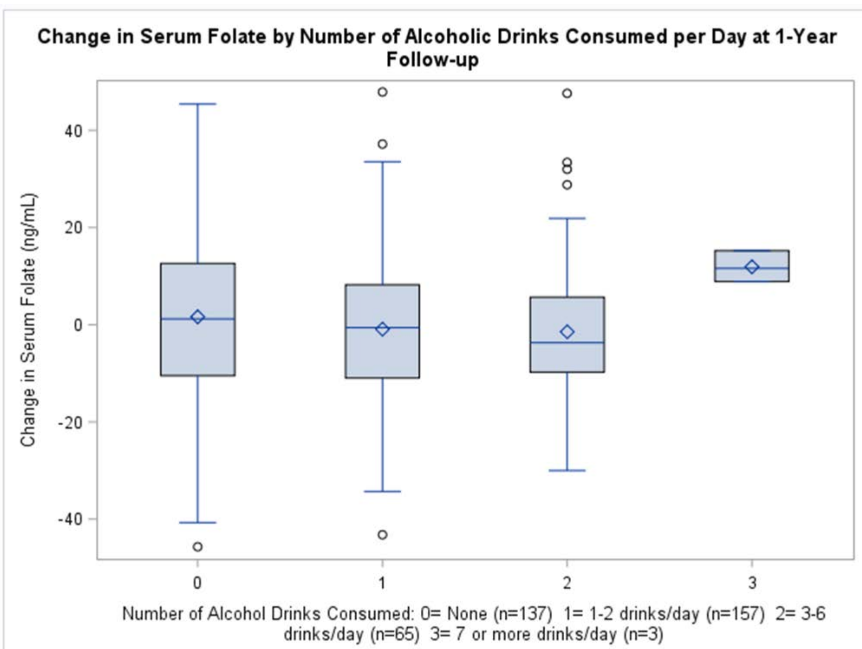


Figure 12 (continued): Box plots of categorical variables and change in serum folate

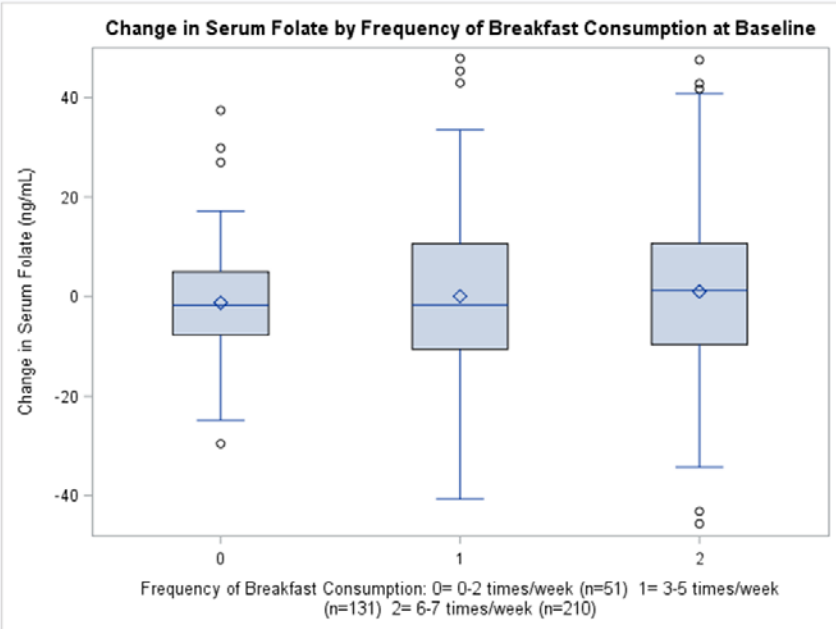


Figure 12 (continued): Box plots of categorical variables and change in serum folate

Table 12 presents the parameter estimates, p-values, and model statistics from the base multivariable model of the association of bariatric surgery and change in serum folate. The reduction in sample size for the model compared to the total sample size (n=413) is due to missing data for individual variables in the model.

Table 12: Multivariable linear regression for change in serum folate – Base Model (n=338)

P-value for the model: 0.0001 **Adjusted R-squared**: 0.11			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-5.9	4.7	0.22
RYGB (vs. LAGB)	11.1	3.2	0.001
Age at surgery (centered at the median)	0.1	0.2	0.44
Black race (vs. white)	-2.5	3.2	0.43
Other race or multiple races (vs. white)	0.4	5.1	0.93
Hispanic ethnicity (vs. non-Hispanic)	-2.7	3.8	0.48
Some college education (vs. high school diploma or less)	3.4	2.4	0.17
College degree or higher (vs. high school diploma or less)	5.9	2.6	0.02
Annual household income: $25,000-$49,999 (vs. <$25,000)	-0.8	2.7	0.76
Annual household income: $50,000-$74,999 (vs. <$25,000)	-4.7	2.8	0.10
Annual household income: ≥$75,000 (vs. <$25,000)	0.6	2.9	0.83
Baseline BMI (centered at the median)	-0.01	0.1	0.92
Percent weight change from baseline	-0.2	0.1	0.09
Daily/weekly MVI use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-1.6	6.0	0.79
Daily/weekly MVI use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	4.7	3.9	0.23
Daily/weekly MVI use baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	0.7	3.7	0.84
Daily/weekly folate supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-8.0	8.2	0.32
Daily/weekly folate supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	8.3	3.6	0.02
Daily/weekly folate supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	-12.6	11.5	0.28

Abbreviations: RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding; BMI: body mass index; MVI: multivitamin

Table 13 presents the parsimonious multivariable model. College degree or higher was removed from the model based on the results of the multiple degree of freedom test for education (p=.40).

Table 13: Multivariable linear regression for change in serum folate – Parsimonious Model (n=354)

P-value for the model: <.0001 **Adjusted R-squared**: 0.10			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-5.7	3.5	0.11
RYGB (vs. LAGB)	6.5	2.1	0.002
Black race (vs. white)	-2.7	3.1	0.38
Daily/weekly MVI use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-2.2	5.8	0.70
Daily/weekly MVI use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	4.1	3.7	0.27
Daily/weekly MVI use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	0.1	3.6	0.97
Daily/weekly folate supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-5.6	8.1	0.47
Daily/weekly folate supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	8.3	3.5	0.02
Daily/weekly folate supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	-14.4	9.4	0.12

Abbreviations: RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding

Only baseline serum folate was significant when added stepwise to the parsimonious model. The final model is presented in Table 14. No significant interactions were identified in the final main effects model. The final model was tested for the assumptions of multivariable linear regression with no violations identified. The residuals were normally distributed, there was no violation of linearity or homoscedasticity, and no evidence of collinearity among the independent variables.

Table 14: Final Model: Association of bariatric surgery and change in serum folate based on multivariable linear regression (n=354)

P-value for the model: <.0001 **Adjusted R-squared**: 0.28			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	8.4	3.3	0.01
RYGB (vs. LAGB)	3.0	1.7	0.08
Black race (vs. white)	-1.5	2.5	0.56
Daily/weekly MVI use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-3.4	5.0	0.50
Daily/weekly MVI use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	7.1	3.3	0.03
Daily/weekly MVI use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	6.6	3.3	0.04
Daily/weekly folate supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-9.1	7.2	0.20
Daily/weekly folate supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	4.8	3.0	0.12
Daily/weekly folate supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	-16.5	8.2	0.045
Baseline serum folate	-0.7	0.1	<.0001

Abbreviations: RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding: MVI: multivitamin

Type of bariatric surgery was not significantly associated with change in serum folate from baseline to one year after surgery when controlling for the other variables in the model (Black race, daily/weekly MVI use, daily/weekly folate supplement use, and baseline serum folate level). The overall clinical site effect was tested using the multiple degree of freedom test and was not significant (p=.55); therefore, clinical sites were not included in the final model.

4.2.3 Association of bariatric surgery and folate deficiency one year after surgery

The subjects' serum folate levels at baseline and one-year follow-up were classified as deficient (<3 ng/mL), within the normal range (3-25 ng/mL), and above the normal range (high) (>25 ng/mL). Table 15 shows deficiency status by surgery separately for baseline and one-year follow-up. Prior to surgery, no subjects who underwent LAGB and only one subject (0.4%) who underwent RYGB were deficient in folate. One year following surgery, only one subject (0.7%) who underwent LAGB and three subjects (1.1%) who underwent RYGB were folate-deficient. The prevalence of folate deficiency at baseline and one year following surgery did not differ significantly by surgery (p=1.0 for both time points). Per self-report, four subjects were pregnant at the one-year postoperative follow-up visit. None of these subjects were deficient in folate at baseline or at one-year follow-up.

Table 15: Folate deficiency status by surgery at baseline and one-year follow-up

	LAGB (n=141)	RYGB (n=272)	P
	n (%)	n (%)	
At baseline:			
Deficient	0 (0)	1 (0.4)	1.00
Not-deficient	141 (100.0)	271 (99.6)	
At 1-year follow-up:			
Deficient	1 (0.7)	3 (1.1)	1.00
Not-deficient	140 (99.3)	269 (98.9)	

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects

Table 16 (LABG group) and Table 17 (RYGB group) present folate status (deficient, within the normal range, or high) at baseline and one-year follow-up. In the LAGB group, one subject (1.6%) whose serum folate was in the normal range at baseline was deficient one year after surgery. Overall, the distributions at baseline and follow-up did not change significantly (p=.18). For the RYGB group, three subjects (1.9%) who were within the normal range prior to RYGB were deficient one year after surgery. The overall distributions changed from pre-op to post-op such that a greater percentage of subjects went from within the normal range to high rather than changing from within the normal range to deficient or from high to within the normal range (p=.01). Prior to surgery, 54.6 % of subjects who underwent LAGB and 41.2% of subjects who underwent RYGB had high serum folate levels. One year after surgery, 44.0% of subjects who underwent LAGB and 52.9% of subjects who underwent RYGB had high serum folate levels.

Table 16: Folate sufficiency status at baseline and one-year postoperative for the LAGB group

Folate Status at Baseline (LAGB)	Folate Status at 1-Year Follow-up (LAGB)			
	Deficient	Normal Range	High	Total (frequency, percent)
Deficient (frequency, row percentage)	0 0%	0 0%	0 0%	0 0%
Normal range (frequency, row percentage)	1 1.6%	42 65.6%	21 32.8%	64 45.4%
High (frequency, row percentage)	0 0%	36 46.8%	41 53.2%	77 54.6%
Total (frequency, percentage)	1 0.7%	78 55.3%	62 44.0%	141 100%

Abbreviations: LAGB: laparoscopic adjustable gastric banding

Table 17: Folate sufficiency status at baseline and one-year postoperative for the RYGB group

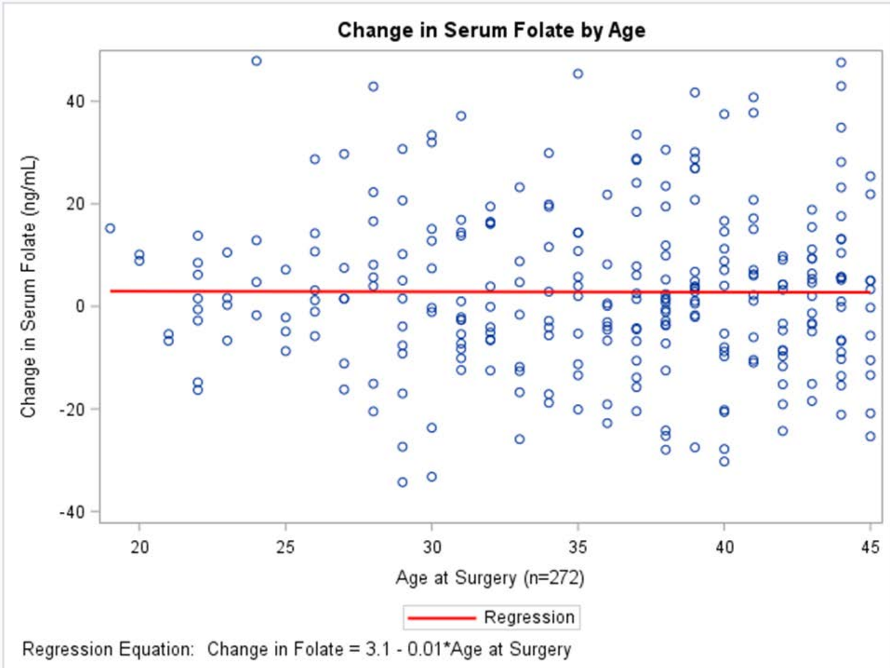
Folate Status at Baseline (RYGB)	Folate Status at 1-Year Follow-up (RYGB)			
	Deficient	Normal Range	High	Total (frequency, percent)
Deficient (frequency, row percentage)	0 0%	1 100%	0 0%	1 0.4%
Normal range (frequency, row percentage)	3 1.9%	93 58.5%	63 39.6%	159 58.4%
High (frequency, row percentage)	0 0%	31 27.7%	81 72.3%	112 41.2%
Total (frequency, percentage)	3 1.1%	125 46.0%	144 52.9%	272 100%

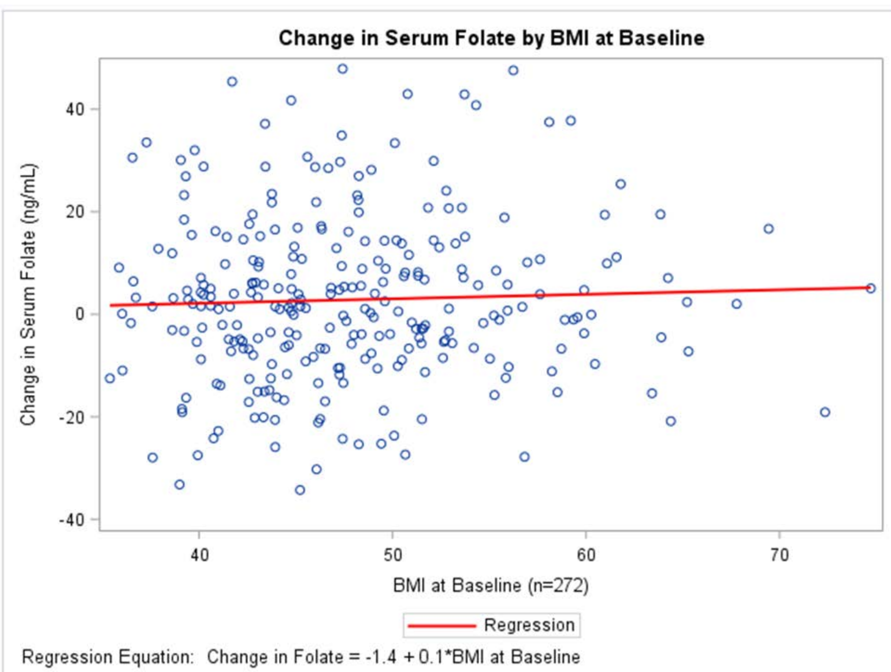
Abbreviations: RYGB: Roux-en-Y gastric bypass

In total, only four subjects (1.0%) were deficient in folate one year after bariatric surgery. Due to the low prevalence of folate deficiency, further analysis using multivariable logistic regression to determine if there was an association between bariatric surgery and folate deficiency was deemed unnecessary.

4.2.4 Factors associated with change in serum folate from baseline to one year after RYGB

Plots of individual covariates versus change in serum folate for subjects who had undergone RYGB are presented in Figure 13 and Figure 14.





<u>Abbreviations</u>: BMI: body mass index

Figure 13: Scatter plots of continuous variables and change in serum folate after RYGB

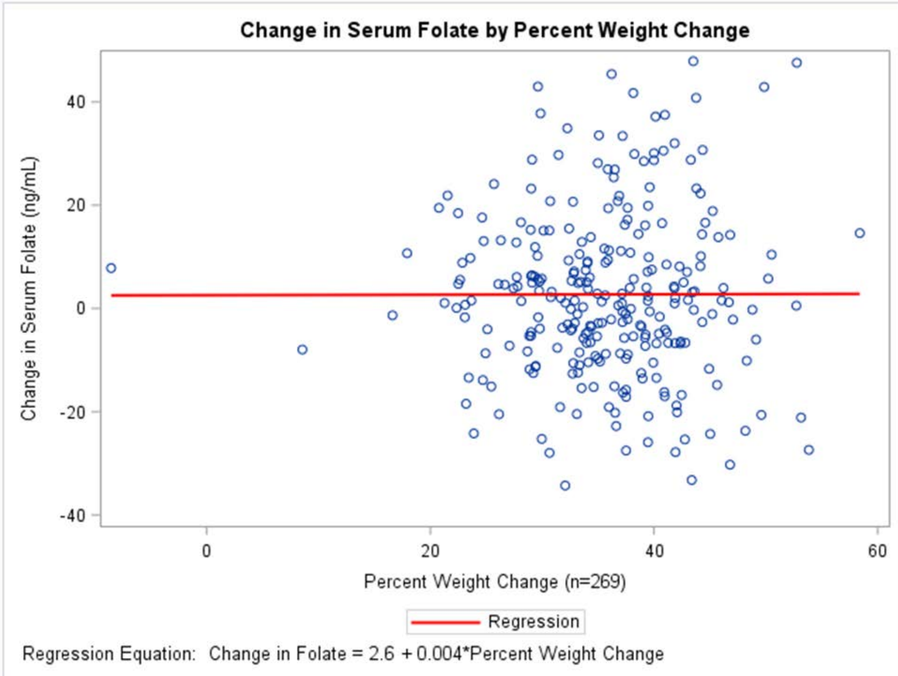


Figure 13 (continued): Scatter plots of continuous variables and change in serum folate after RYGB

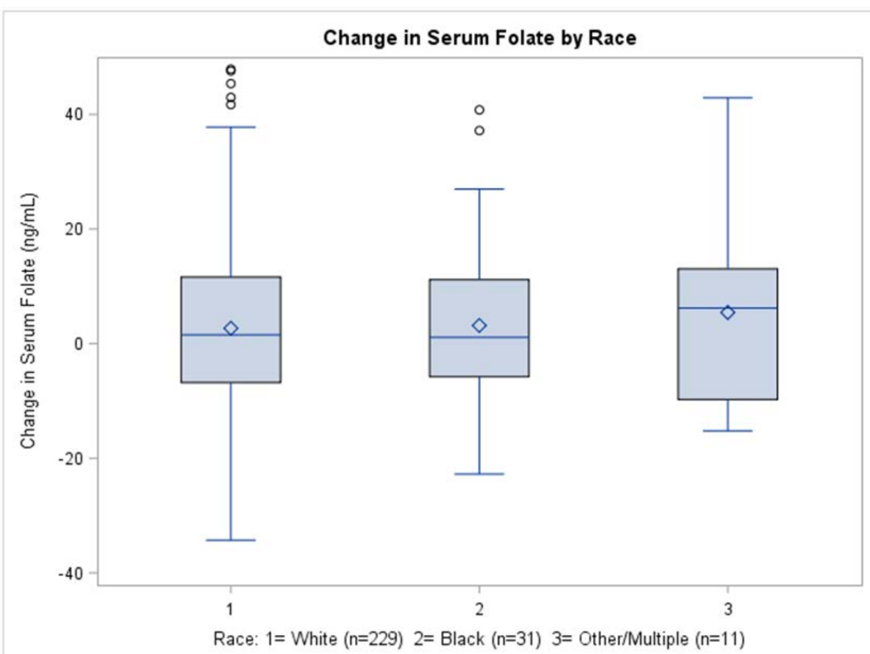


Figure 14: Box plots of categorical variables and change in serum folate after RYGB

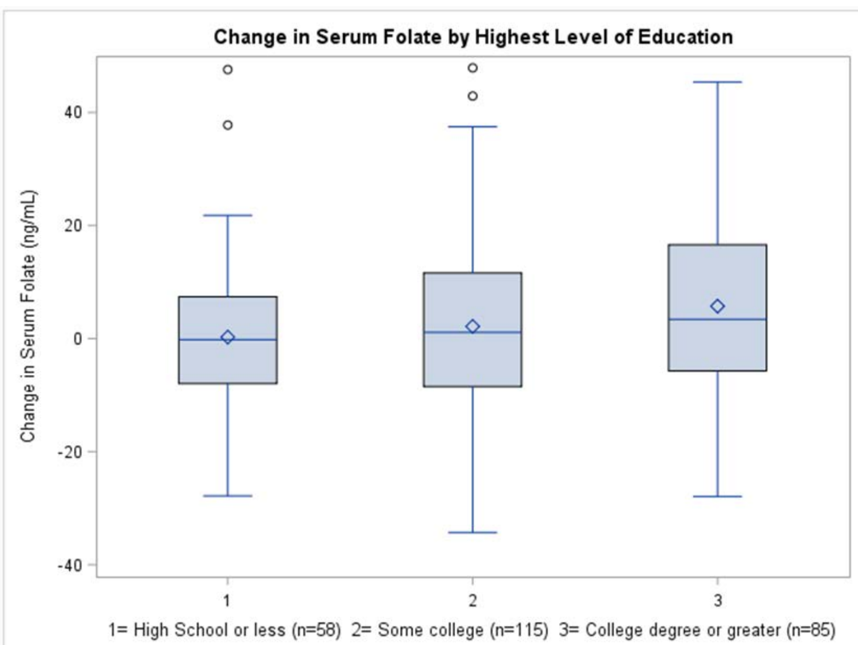
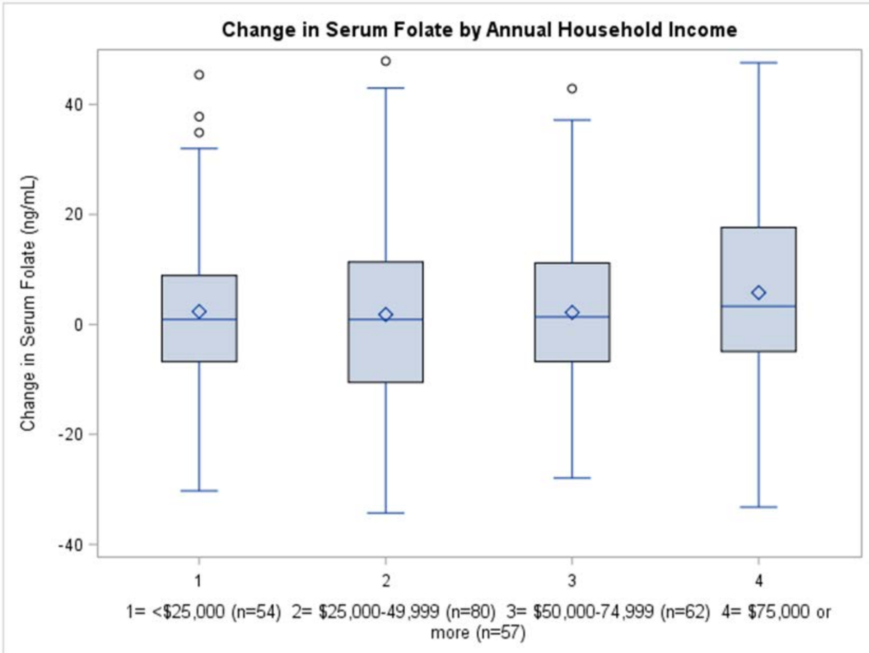
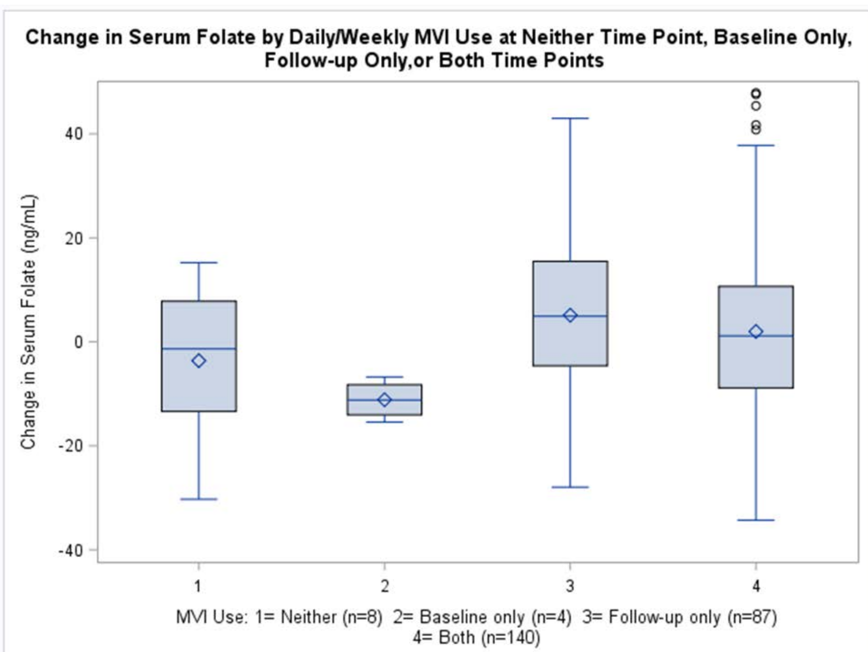


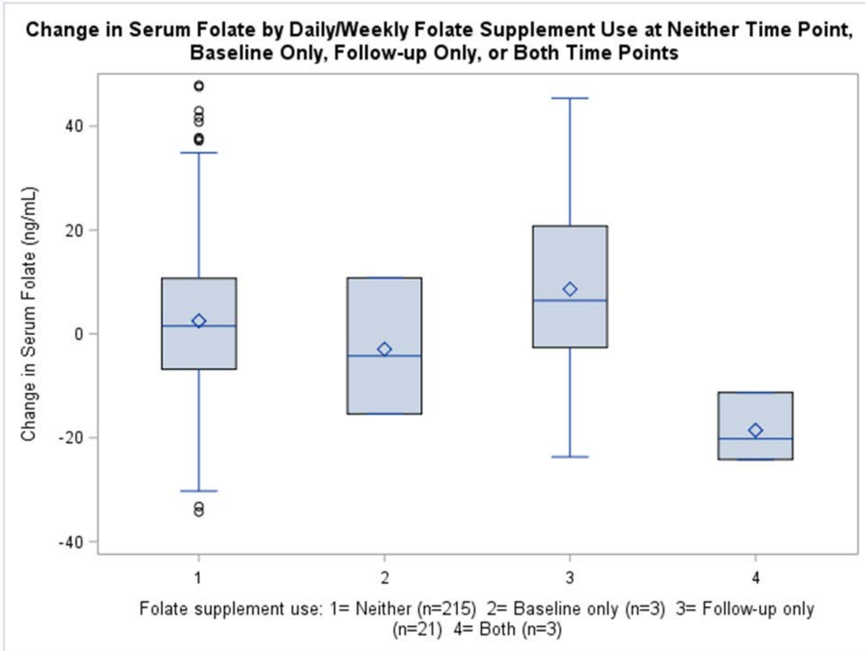
Figure 14 (continued): Box plots of categorical variables and change in serum folate after RYGB





<u>Abbreviations</u>: MVI: multivitamin

Figure 14 (continued): Box plots of categorical variables and change in serum folate after RYGB



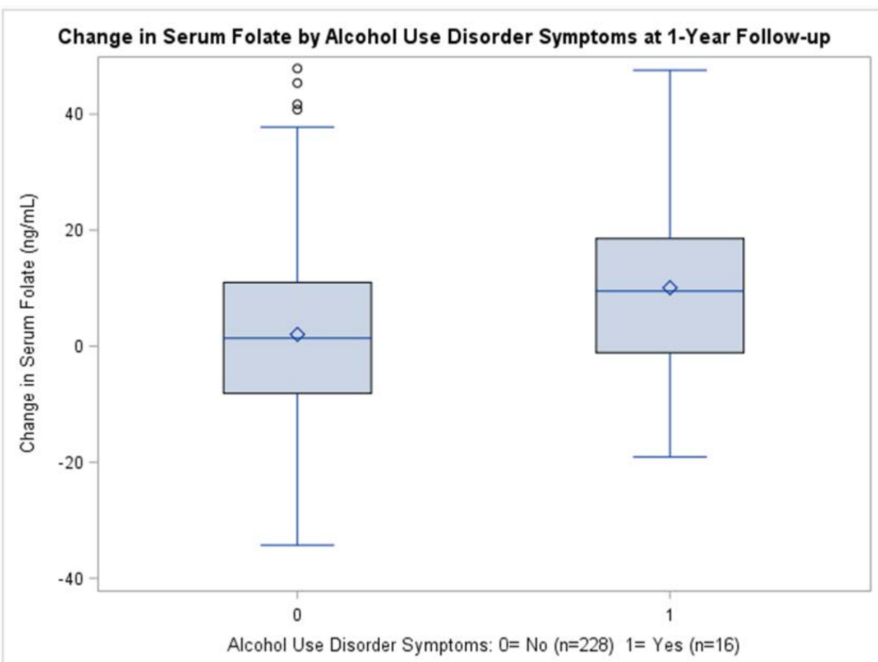
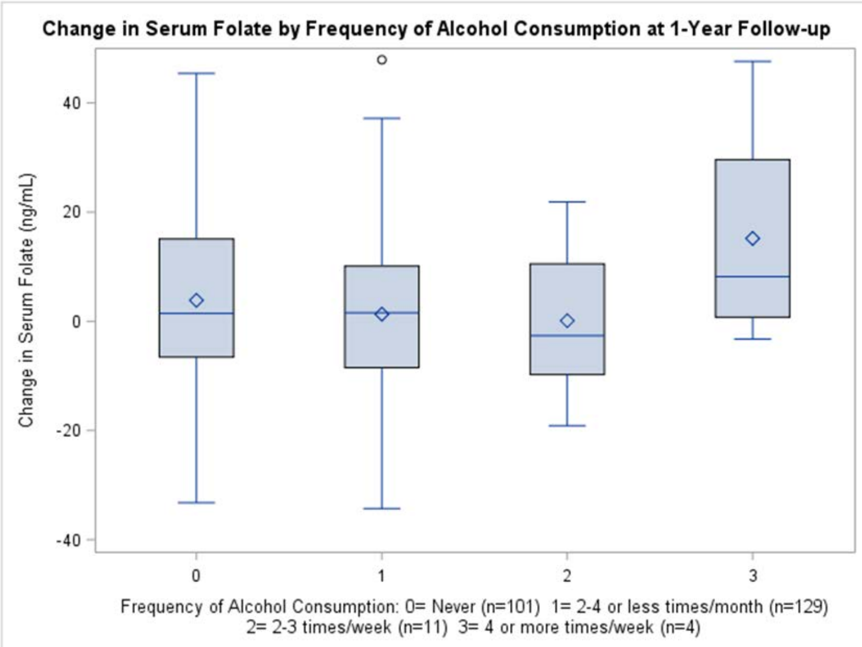


Figure 14 (continued): Box plots of categorical variables and change in serum folate after RYGB



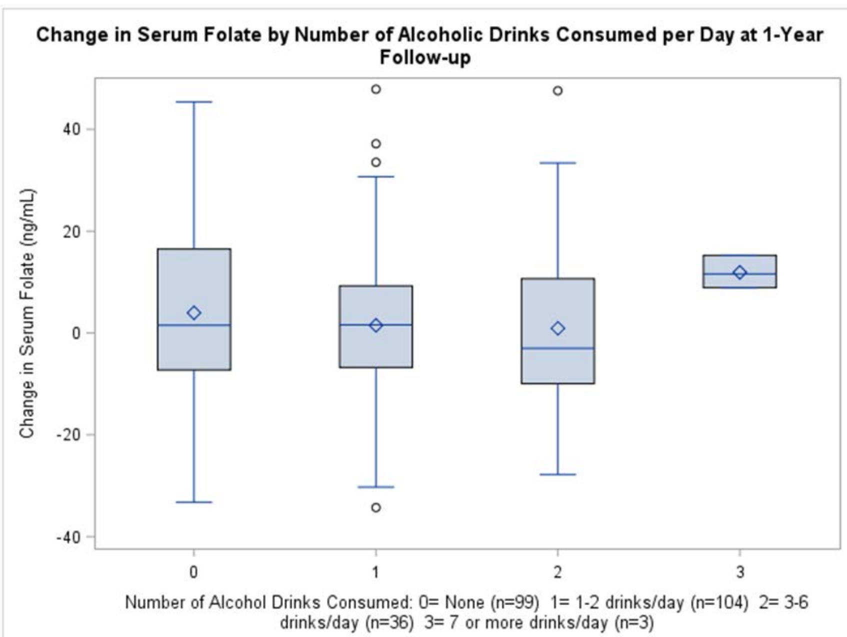


Figure 14 (continued): Box plots of categorical variables and change in serum folate after RYGB

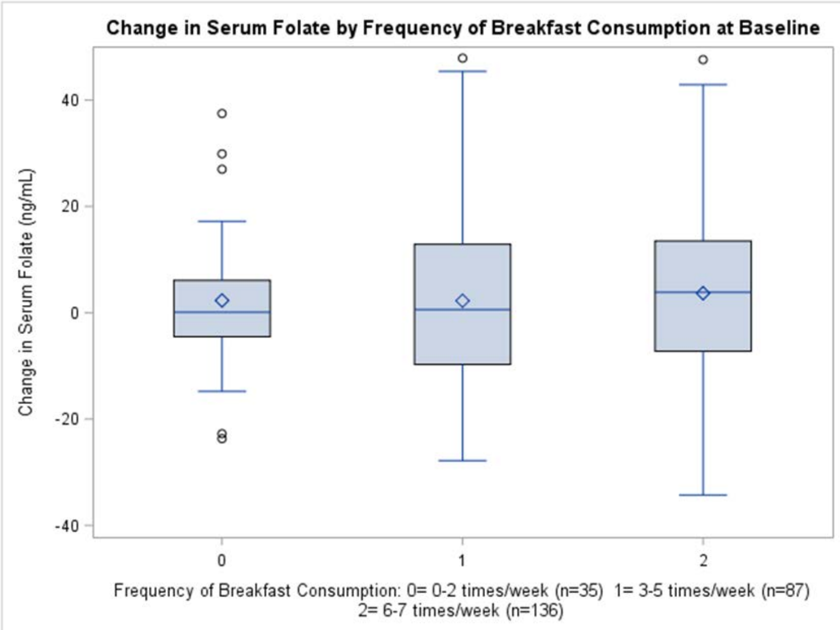


Figure 14 (continued): Box plots of categorical variables and change in serum folate after RYGB

Parameter estimates, p-values, and model statistics for the base multivariable model of factors associated with change in serum folate among women who had undergone RYGB are presented in Table 18. The reduction in sample size for the model compared to total sample size (n=272) is due to missing data for individual variables.

Table 18: Multivariable linear regression model for change in serum folate after RYGB – Base Model (n=228)

P-value for the model: 0.10 **Adjusted R-squared**: 0.04			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-1.0	9.2	0.91
Age at surgery (centered at the median)	0.1	0.2	0.58
Black race (vs. white)	-3.0	3.8	0.43
Other race or multiple races (vs. white)	-1.7	6.0	0.78
Hispanic ethnicity (vs. non-Hispanic)	-4.0	5.1	0.44
Some college education (vs. high school diploma or less)	2.6	2.9	0.38
College degree or higher (vs. high school diploma or less)	5.9	3.3	0.07
Annual household income: $25,000-$49,999 (vs. <$25,000)	-0.8	3.2	0.80
Annual household income: $50,000-$74,999 (vs. <$25,000)	-2.9	3.6	0.43
Annual household income: ≥$75,000 (vs. <$25,000)	-0.7	3.7	0.85
Baseline BMI (centered at the median)	0.1	0.2	0.70
Percent weight change from baseline	-0.2	0.2	0.30
Daily/weekly MVI use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-4.0	11.1	0.72
Daily/weekly MVI use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	10.5	6.5	0.11
Daily/weekly MVI use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	7.6	6.4	0.24
Daily/weekly folate supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-0.9	10.1	0.93
Daily/weekly folate supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	8.2	3.9	0.04
Daily/weekly folate supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	-13.7	12.0	0.25

Abbreviations: BMI: body mass index; MVI: multivitamin

Only daily/weekly folate supplement use at follow-up only was significant in the base model. No variables were significant in the simple regression or multiple degree of freedom tests. Table 19 shows the parsimonious multivariable model.

Table 19: Multivariable linear regression model for change in serum folate after RYGB – Parsimonious Model (n=238)

P-value for the model: 0.003 **Adjusted R-squared**: 0.08			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-3.2	6.0	0.60
Black race (vs. white)	-2.7	3.5	0.44
Daily/weekly MVI use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-4.0	10.4	0.70
Daily/weekly MVI use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	9.0	5.9	0.13
Daily/weekly MVI use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	6.3	5.8	0.28
Daily/weekly folate supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	1.3	9.7	0.89
Daily/weekly folate supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	9.0	3.8	0.02
Daily/weekly folate supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	-11.6	9.6	0.23

Abbreviations: MVI: multivitamin

When all covariates were added to the model stepwise, the only variable that had a $p<.05$ was baseline serum folate. The final model is presented in Table 20. No significant interactions were identified in the final main effects model. The final model met the assumptions of multivariable linear regression. The residuals for the model were normally distributed. There was no evidence of heteroscedasticity, non-linearity between the dependent and independent variables, or collinearity among the independent variables in the final model.

Table 20: Final Model: Factors associated with change in serum folate from baseline to one year following RYGB among women of childbearing age (n=238)

P-value for the model: <.0001 **Adjusted R-squared**: 0.24			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	6.9	5.3	0.20
Black race (vs. white)	-1.7	3.0	0.58
Daily/weekly MVI use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-4.7	9.1	0.60
Daily/weekly MVI use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	10.9	5.3	0.04
Daily/weekly MVI use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	12.3	5.3	0.02
Daily/weekly folate supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-5.9	8.7	0.50
Daily/weekly folate supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	4.5	3.3	0.18
Daily/weekly folate supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	-17.5	8.4	0.04
Baseline serum folate	-0.7	0.1	<.0001

Abbreviations: MVI: multivitamin

For women of childbearing age who had undergone RYGB, daily/weekly MVI use at follow-up only and daily/weekly MVI use at both baseline and follow-up were negative confounders of the relationship between RYGB and change in serum folate. Daily/weekly MVI use at follow-up only was associated, on average, with a 10.9 ng/mL greater change in serum folate compared to subjects who reported not taking a MVI on a daily or weekly basis at either baseline or one-year follow-up (p=.04), after adjusting for the other variables in the model. Daily/weekly MVI use at both baseline and follow-up was associated, on average, with a 12.3 ng/mL greater change in serum folate compared to subjects who reported not taking a MVI on a daily or weekly basis at either baseline or one-year follow-up (p=.02), after adjusting for the other variables in the model. Daily/weekly use of a folate supplement at both baseline and follow-up was associated, on average, with a 17.5 ng/mL lower change in serum folate compared to subjects who reported not taking a folate supplements on a daily or weekly basis at either baseline or one-year follow-up (p=.04), after adjusting for the other variables in the model. Folate supplements are not standardly recommended after RYGB; and therefore, folate supplement use would not be considered a confounder of the relationship. Baseline serum folate level was significantly (p<.0001) associated with change in serum folate, after adjusting for the other covariates in the model. The overall clinical site effect was tested using the multiple degree of freedom test and was not significant (p=.31); therefore, clinical sites were not included in the final model.

4.3 FERRITIN

4.3.1 Serum ferritin levels at baseline and follow-up and change in serum ferritin

Baseline and one-year postoperative serum ferritin levels were available for all 426 subjects. Table 21 presents descriptive statistics for baseline serum ferritin by surgery. Median values for LAGB and RYGB are 51.0 ng/mL and 59.0 ng/mL, respectively, with ranges from 2.0-441.0 ng/mL among women who underwent LAGB and 1.5-320.0 ng/mL among those who underwent RYGB. The normal range for serum ferritin for adult females is 10-150 ng/mL,[55] and iron deficiency has been defined as <15 ng/mL for adult females.[79,81] A serum ferritin level of <15 ng/mL was considered below the normal range (deficient), a serum ferritin level between 15-150 ng/mL was considered within the normal range, and a level >150 ng/mL was classified as above the normal range (high).

Table 21: Serum ferritin at baseline

	Median (ng/mL)	25th %ile (ng/mL)	75th %ile (ng/mL)	Range (ng/mL)	Below the normal range: n (%)	Within the normal range: n (%)	Above the normal range: n (%)
LAGB (n=146)	51.0	26.0	92.0	2.0-441.0	12 (8.2%)	120 (82.2%)	14 (9.6%)
RYGB (n=280)	59.0	33.0	95.5	1.5-320.0	19 (6.8%)	231 (82.5%)	30 (10.7%)

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; %ile: percentile

Table 22 presents descriptive statistics for serum ferritin at the one-year postoperative visit by surgery. Median values for LAGB and RYGB were 46.0 ng/mL and 43.0 ng/mL, respectively, with ranges from 1.1-464.0 ng/mL among women who underwent LAGB and 1.8-435.0 ng/mL among those who underwent RYGB surgery.

Table 22: Serum ferritin at the one-year postoperative visit

	Median (ng/mL)	25th %ile (ng/mL)	75th %ile (ng/mL)	Range (ng/mL)	Below the normal range: n (%)	Within the normal range: n (%)	Above the normal range: n (%)
LAGB (n=146)	46.0	26.0	84.0	1.1–464.0	20 (13.7%)	108 (74.0%)	18 (12.3%)
RYGB (n=280)	43.0	18.0	83.5	1.8–435.0	61 (21.8%)	194 (69.3%)	25 (8.9%)

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; %ile: percentile

The change in serum ferritin was calculated by subtracting the baseline serum level from the one-year postoperative visit serum level. A negative value indicates a decrease in ferritin level from baseline to one-year after surgery. Descriptive statistics are presented in Table 23. The mean change for subjects who underwent LAGB was a decrease of 3.8 ng/mL with a range from a decrease of 156.0 ng/mL to an increase of 130.0 ng/mL. For subjects who underwent

RYGB, the mean change was a decrease of 11.9 ng/mL with a range from a decrease of 192.0 ng/mL to an increase of 249.0 ng/mL

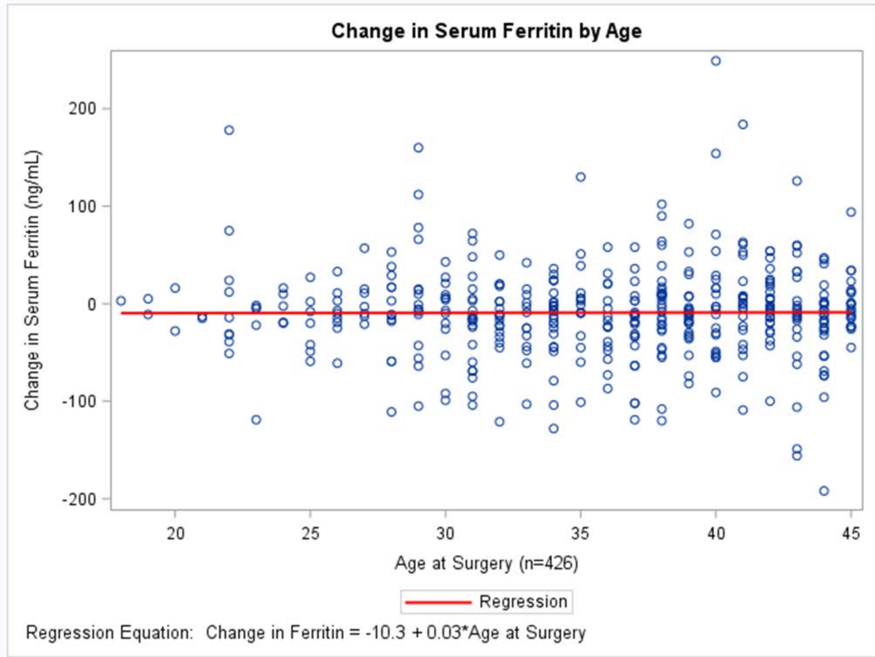
Table 23: Change in serum ferritin level from baseline to the one-year postoperative visit

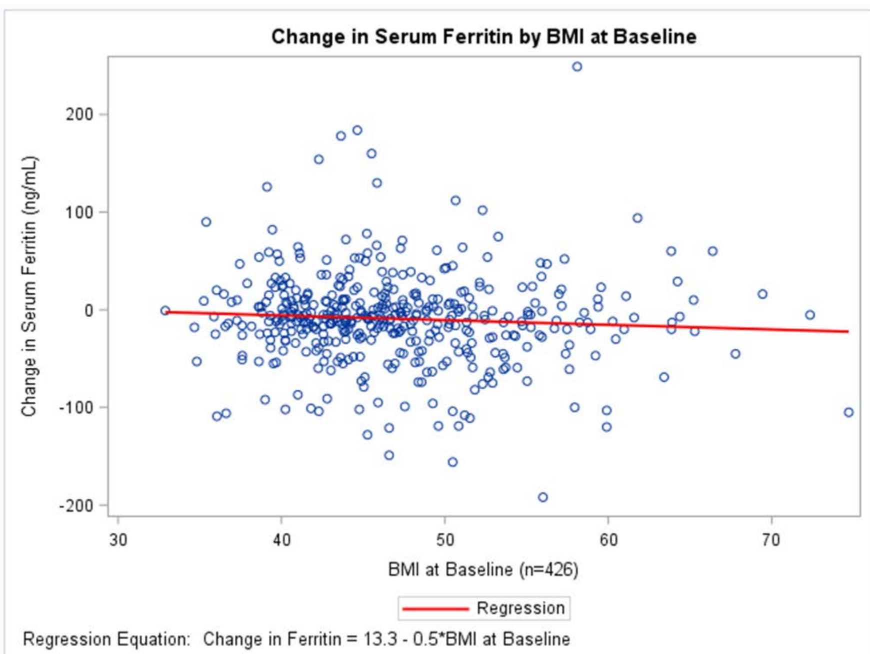
	Mean (ng/mL)	SD	25th %ile (ng/mL)	Median (ng/mL)	75th %ile (ng/mL)	Range (ng/mL)
LAGB (n=146)	-3.8	38.5	-20.0	-4.0	12.0	-156.0 to 130.0
RYGB (n=280)	-11.9	51.3	-35.0	-12.0	9.0	-192.0 to 249.0

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects; SD: standard deviation; %ile: percentile

4.3.2 Association of bariatric surgery and change in serum ferritin

Plots of individual covariates versus change in serum ferritin are presented in Figure 15 and Figure 16.





Abbreviations: BMI: body mass index

Figure 15: Scatter plots of continuous variables and change in serum ferritin

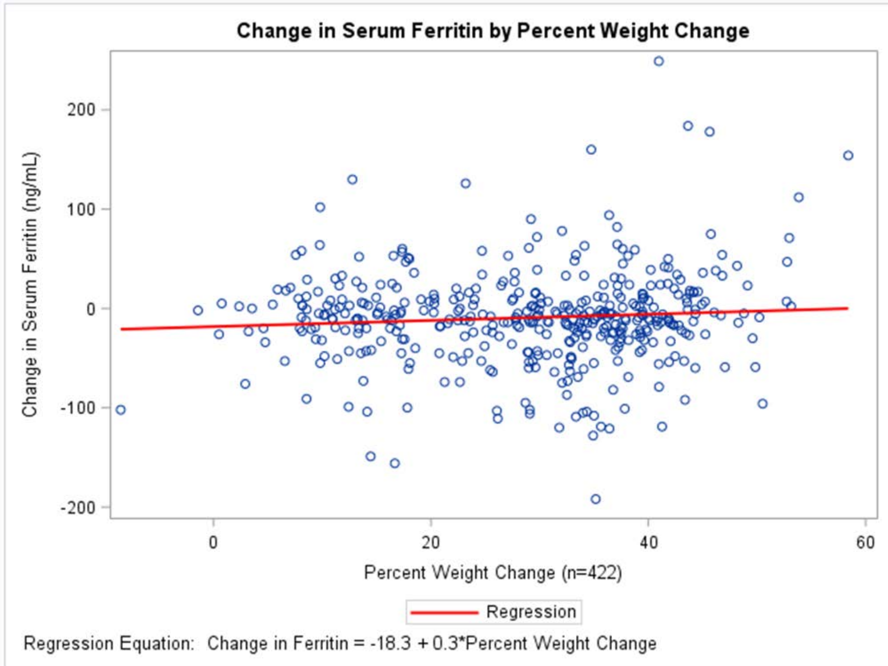
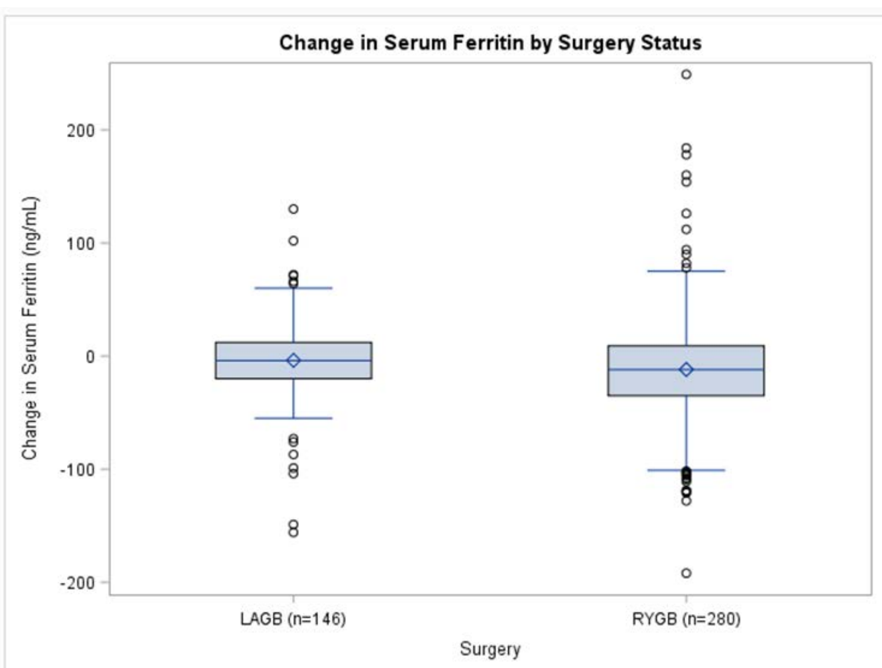
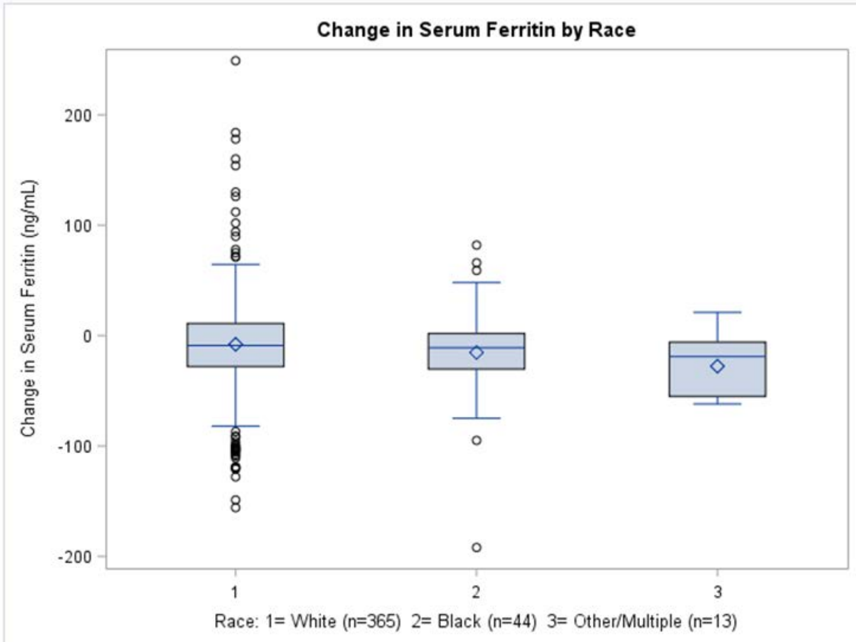


Figure 15 (continued): Scatter plots of continuous variables and change in serum ferritin



Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass

Figure 16: Box plots of categorical variables and change in serum ferritin



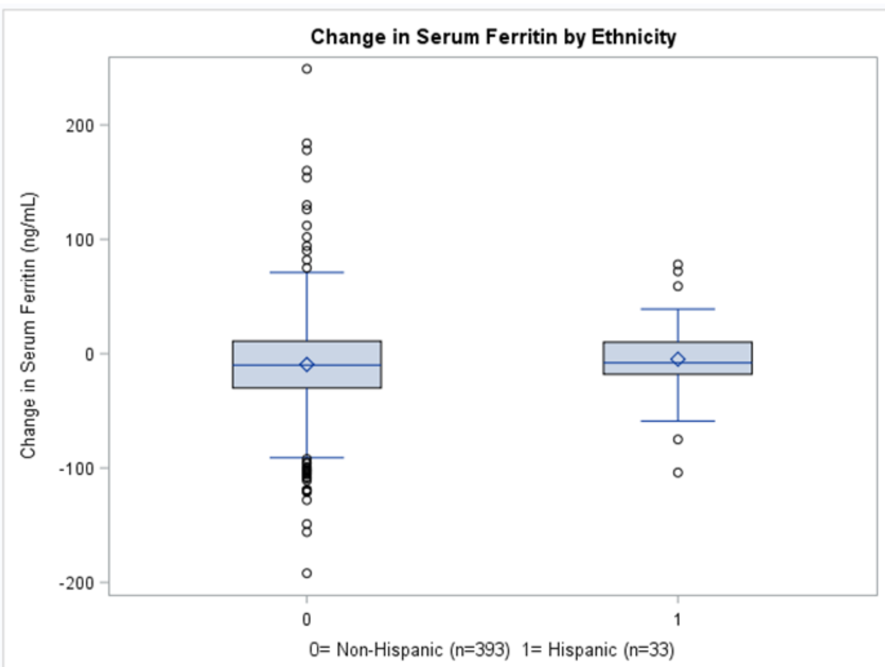
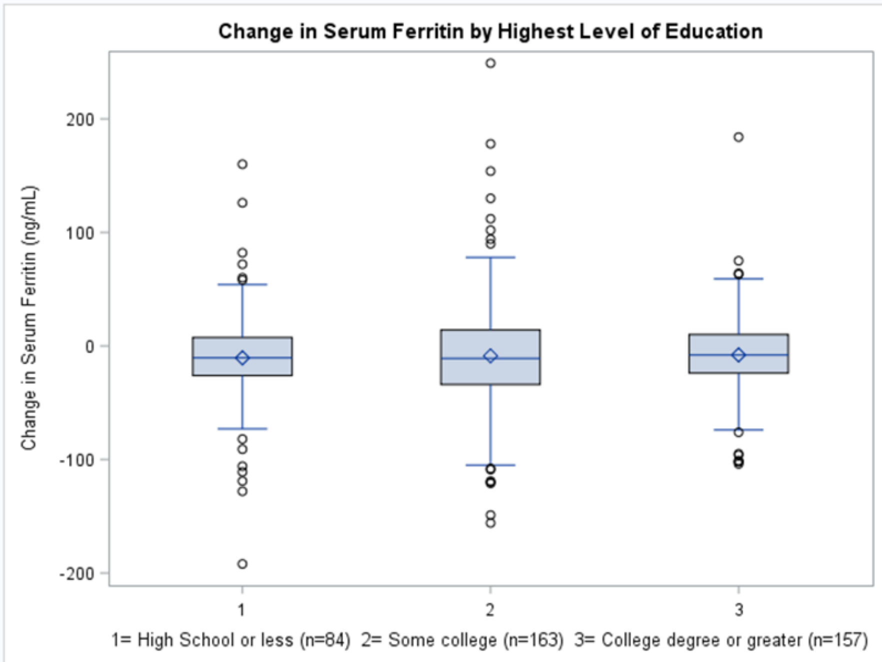


Figure 16 (continued): Box plots of categorical variables and change in serum ferritin



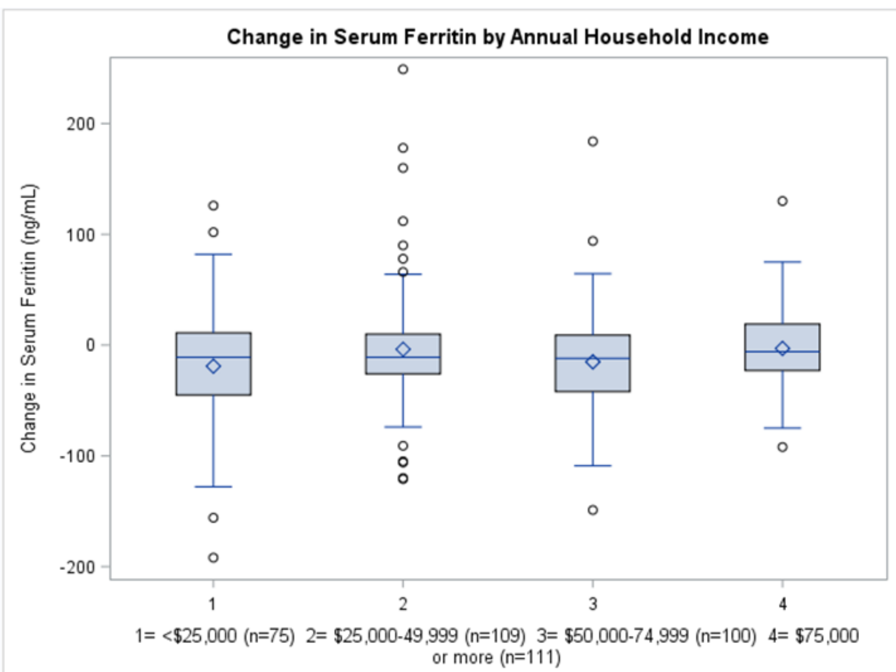
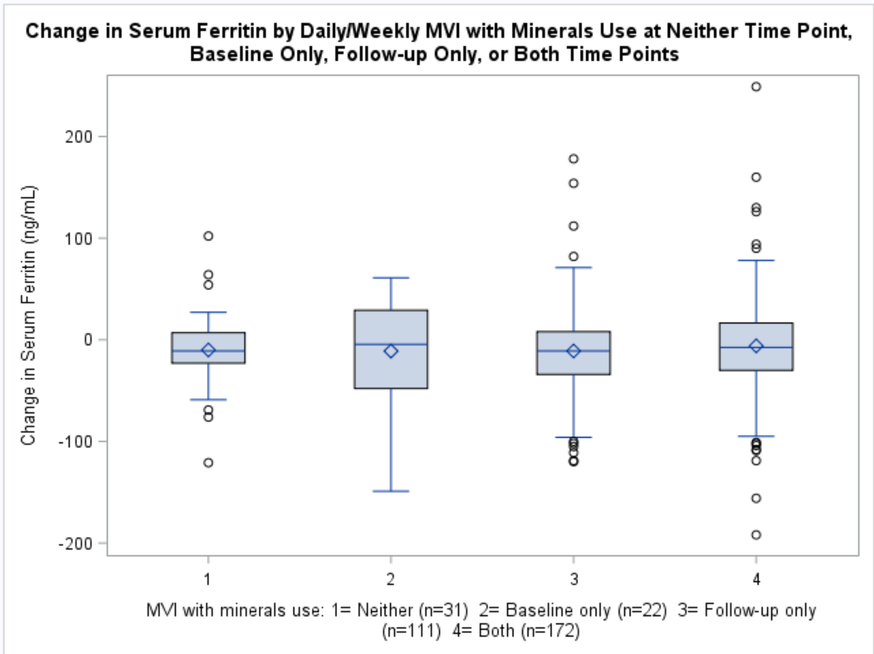


Figure 16 (continued): Box plots of categorical variables and change in serum ferritin



Abbreviations: MVI: multivitamin

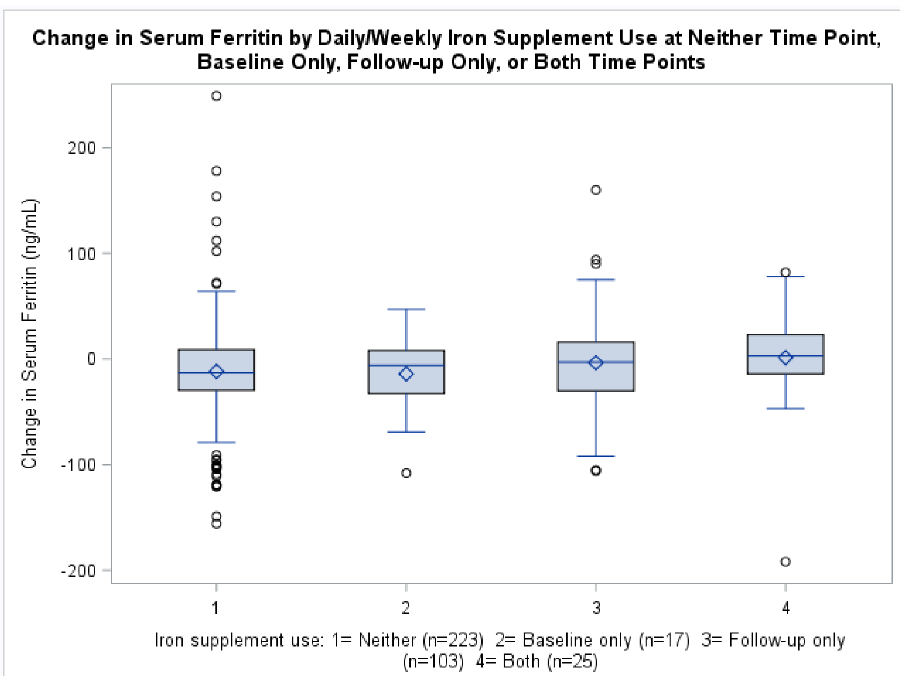


Figure 16 (continued): Box plots of categorical variables and change in serum ferritin

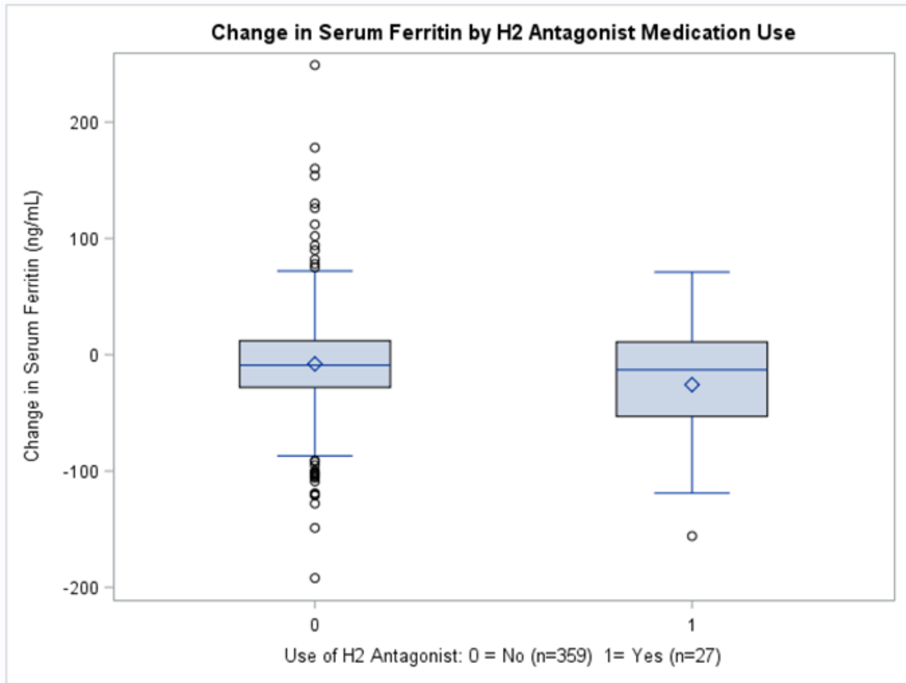


Figure 16 (continued): Box plots of categorical variables and change in serum ferritin

Parameter estimates, p-values, and model statistics from the base multivariable linear regression model for association of bariatric surgery with change in serum ferritin are presented in Table 24. The reduction in sample size for the model (compared to n=426) is due to missing data for individual variables.

Table 24: Multivariable linear regression for change in serum ferritin – Base Model (n=313)

P-value for the model: 0.01
Adjusted R-squared: 0.07

Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-34.1	14.1	0.02
RYGB (vs. LAGB)	-38.0	10.2	0.0002
Age at surgery (centered at the median)	0.1	0.5	0.79
Black race (vs. white)	-10.6	9.9	0.28
Other race or multiple races (vs. white)	-5.5	16.3	0.73
Hispanic ethnicity (vs. non-Hispanic)	1.2	11.8	0.92
Some college education (vs. high school diploma or less)	-0.4	7.8	0.96
College degree or higher (vs. high school diploma or less)	-5.9	8.2	0.47
Annual household income: $25,000-$49,999 (vs. <$25,000)	18.3	8.4	0.03
Annual household income: $50,000-$74,999 (vs. <$25,000)	-4.0	8.9	0.65
Annual household income: ≥$75,000 (vs. <$25,000)	11.8	9.0	0.19
Baseline BMI (centered at the median)	-0.4	0.4	0.39
Percent weight change from baseline	1.2	0.4	0.002
Daily/weekly MVI with minerals use at baseline only (vs. no daily/weekly use at baseline or follow-up)	0.6	14.5	0.97
Daily/weekly MVI with minerals use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	0.8	10.9	0.94
Daily/weekly MVI with minerals use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	1.0	10.3	0.92
Daily/weekly iron supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	1.1	13.1	0.93
Daily/weekly iron supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	13.2	7.2	0.07
Daily/weekly iron supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	25.5	11.6	0.03

Abbreviations: RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding; BMI: body mass index; MVI: multivitamin

Table 25 shows the parsimonious multivariable model. Annual household income was added to the model based on the statistical significance of the multiple degree of freedom test (p=.04) in the simple linear model.

Table 25: Multivariable linear regression for change in serum ferritin – Parsimonious Model (n=314)

P-value for the model: 0.001 **Adjusted R-squared**: 0.08			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-35.7	12.9	0.01
RYGB (vs. LAGB)	-38.3	9.9	0.0001
Black race (vs. white)	-10.4	9.8	0.29
Annual household income: $25,000-$49,999 (vs. <$25,000)	17.4	8.2	0.04
Annual household income: $50,000-$74,999 (vs. <$25,000)	-4.8	8.4	0.57
Annual household income: $\geq$$75,000 (vs. <$25,000)	10.3	8.3	0.22
Percent weight change from baseline	1.2	0.4	0.001
Daily/weekly MVI with minerals use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-1.1	14.2	0.94
Daily/weekly MVI with minerals use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	0.03	10.6	1.0
Daily/weekly MVI with minerals use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	0.3	10.2	0.98
Daily/weekly iron supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	0.1	12.9	1.0
Daily/weekly iron supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	13.1	7.2	0.07
Daily/weekly iron supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	24.8	11.4	0.03

Abbreviations: RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding; MVI: multivitamin

When adding the other covariates to the parsimonious model stepwise, only baseline serum ferritin had a p<.05. Household income was no longer significant and was removed from the model. The overall clinical site effect was tested using the multiple degree of freedom test and was not significant (p=.29); therefore, clinical sites were removed from the final model. The final model is presented in Table 26. The assumptions for multivariable linear regression were tested for the final model. The residuals for the model were normally distributed. There was no violation of homoscedasticity or linearity, and no collinearity was identified among the independent variables.

Table 26: Final Model: Association of bariatric surgery and change in serum ferritin level based on multivariable linear regression (n=325)

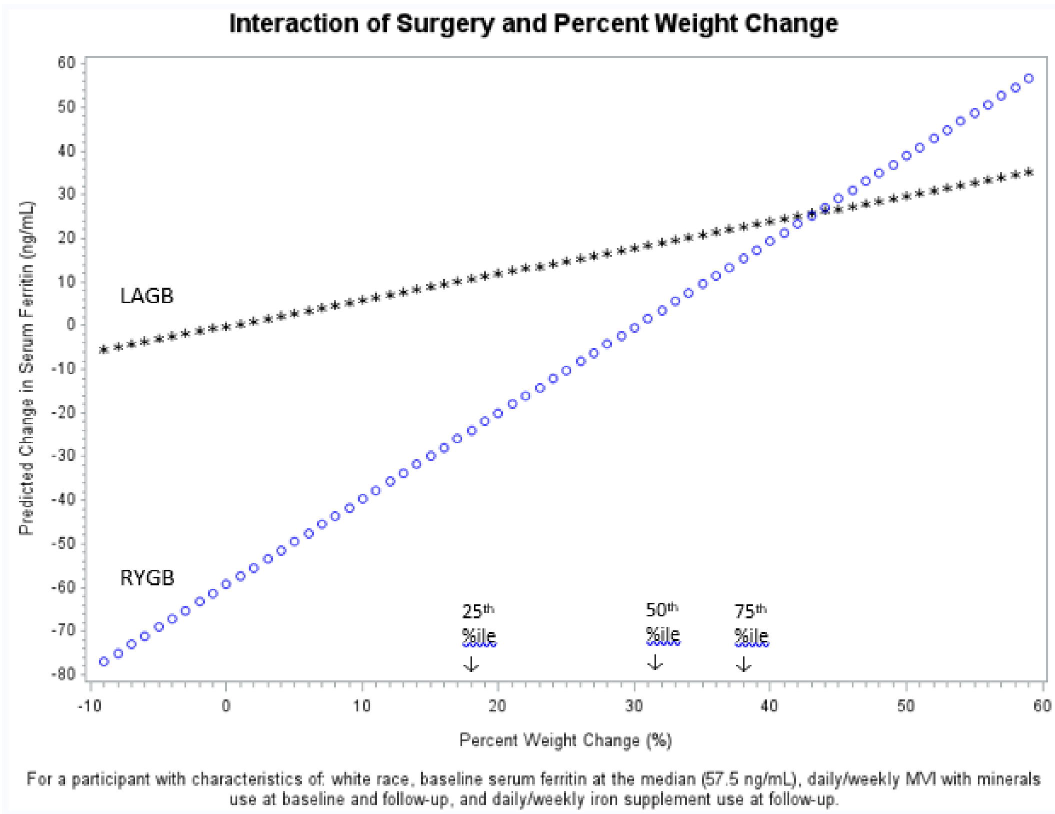
P-value for the model: <.0001 **Adjusted R-squared**: 0.23			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-1.2	12.0	0.92
RYGB (vs. LAGB)*^	-59.3	17.3	0.001
Black race (vs. white)	-6.2	8.0	0.44
Percent weight change from baseline*	0.6	0.5	0.25
Daily/weekly MVI with minerals use at baseline only (vs. no daily/weekly use at baseline or follow-up)	8.3	12.4	0.51
Daily/weekly MVI with minerals use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	0.7	9.4	0.94
Daily/weekly MVI with minerals use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	7.2	9.0	0.42
Daily/weekly iron supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-6.9	11.8	0.56
Daily/weekly iron supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	5.1	6.0	0.40
Daily/weekly iron supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	13.6	10.1	0.18
Baseline serum ferritin^	-0.2	0.1	0.001
*Interaction of type of surgery and percent weight change from baseline	1.4	0.6	0.03
^Interaction of type of surgery and baseline serum ferritin	-0.2	0.1	0.02

Abbreviations: RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding; MVI: multivitamin

The type of bariatric surgery was significantly (p=.001) associated with change in serum ferritin from baseline to one year following surgery, after adjusting for the covariates in the model, such that women who had undergone RYGB had a lower change in serum ferritin than women who had undergone LAGB. There were significant interactions between type of surgery with both percent weight change from baseline (p=0.03) and baseline serum ferritin level (p=0.02), such that the difference in change in serum ferritin between RYGB and LAGB was less pronounced with greater weight loss and more pronounced with higher baseline ferritin level.

4.3.2.1 How surgical procedure moderates associations

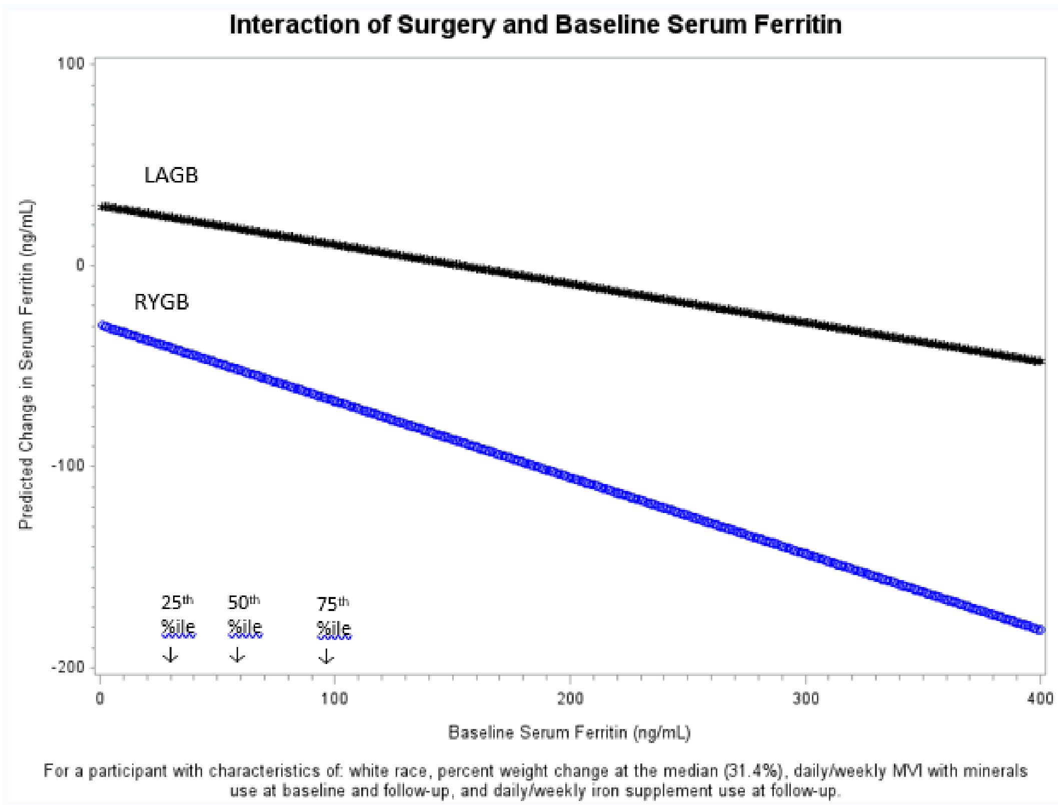
The association between percent weight change and change in serum ferritin is moderated by type of surgery. To illustrate this interaction, the plot in Figure 17 was constructed for predicted change in serum ferritin based on the final regression model (Table 26) with independent variables fixed at the following values: white race, reported daily/weekly MVI with minerals use at baseline and follow-up, reported daily/weekly iron supplement use at follow-up, and baseline serum ferritin at the median level (57.5 ng/mL) for both surgical procedures. The results in Figure 17 indicate that while for both surgical procedures, with greater percent weight change (i.e. more weight loss or less weight gain) there is a greater change in serum ferritin (i.e. greater decrease or smaller increase in serum ferritin from baseline to the one-year post-operative visit). In the case of RYGB, the predicted change in serum ferritin goes from a decrease to an increase above 30.2% weight change. For LAGB, the predicted change in serum ferritin goes from a decrease to an increase above 0.05% weight change. The interaction shows that the increase in serum ferritin with more percent weight loss was more pronounced among women who had undergone RYGB compared to LAGB.



Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; %ile: percentile; MVI: multivitamin
Note: Change in serum ferritin was determined by subtracting the baseline serum level from the one-year postoperative serum level. Percent weight change was calculated as weight at baseline minus weight at the one-year postoperative follow-up visit divided by weight at baseline, and multiplied by 100. A positive value for percent weight change indicates weight loss and a negative value indicates weight gain.

Figure 17: Interaction of Surgery and Percent Weight Change

The association between baseline serum ferritin level and change in serum ferritin is also moderated by type of surgery. Figure 18 shows a plot of predicted change in serum ferritin using the variables included in the final regression model fixed at the following values: white race, percent weight change from baseline at the median (31.4%), reported daily/weekly MVI with minerals use at baseline and follow-up, and reported daily/weekly iron supplement use at follow-up for both surgical procedures. Figure 18 shows that while participants with higher baseline serum ferritin levels have smaller predicted increases or larger predicted decreases in serum ferritin, the predicted change in serum ferritin was more pronounced among the women who had undergone RYGB compared to LAGB.



Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; %ile: percentile; MVI: multivitamin

Note: Change in serum ferritin was determined by subtracting the baseline serum level from the one-year postoperative serum level.

Figure 18: Interaction of Surgery and Baseline Serum Ferritin

4.3.3 Association of bariatric surgery and iron deficiency

The subjects' serum levels at baseline and one-year follow-up were classified as deficient (<15 ng/mL), within the normal range (15-150 ng/mL), and above the normal range/high (>150 ng/mL). Table 27 shows deficiency status by surgery, separately for baseline and follow-up. Prior to surgery, 8.2% of subjects who underwent LAGB and 6.8% of subjects who underwent RYGB were iron-deficient, based on serum ferritin level. The difference in prevalence of deficiency at baseline by surgery was not significant. One year after surgery, there was a significantly (p=.04) smaller percentage of LAGB subjects who were iron deficient (13.7%) compared RYGB subjects (21.8%). Of the four subjects who reported being pregnant at the one-year postoperative follow-up visit, none were deficient at baseline and one subject was iron-deficient at one-year follow-up.

Table 27: Ferritin deficiency status by surgery at baseline and one-year follow-up

	LAGB (n=146)	RYGB (n=280)	P
	n (%)	n (%)	
At baseline:			
Deficient	12 (8.2)	19 (6.8)	0.59
Not-deficient	134 (91.8)	261 (93.2)	
At 1-year follow-up:			
Deficient	20 (13.7)	61 (21.8)	0.04
Not-deficient	126 (86.3)	219 (78.2)	

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass; n: number of subjects

Ferritin status (deficient, within the normal range, or high) at both time points are presented in Table 28 (LAGB group) and Table 29 (RYGB group). In the LAGB group, 9.2% of subjects whose serum ferritin was in the normal range at baseline were deficient one year after surgery. Overall, the distributions at baseline and follow-up did not change significantly (p=.10). For subjects who underwent RYGB, 20.8% of subjects who were within the normal range prior to surgery were iron-deficient one year following surgery and the overall distributions changed pre- to post-surgery such that a greater percentage of subjects went from the normal range to deficient or high to the normal range rather than changing from deficient to the normal range or the normal range to high (p<.0001).

Table 28: Ferritin sufficiency status at baseline and one-year postoperative for the LAGB group

Ferritin Status at Baseline (LAGB)	Ferritin Status at 1-Year Follow-up (LAGB)			
	Deficient	Normal Range	High	Total (frequency, percent)
Deficient (frequency, row percentage)	9 75.0%	3 25.0%	0 0%	12 8.2%
Normal range (frequency, row percentage)	11 9.2%	102 8.0%	7 5.8%	120 82.2%
High (frequency, row percentage)	0 0%	3 21.4%	11 78.6%	14 9.6%
Total (frequency, percentage)	20 13.7%	108 74.0%	18 12.3%	146 100.0%

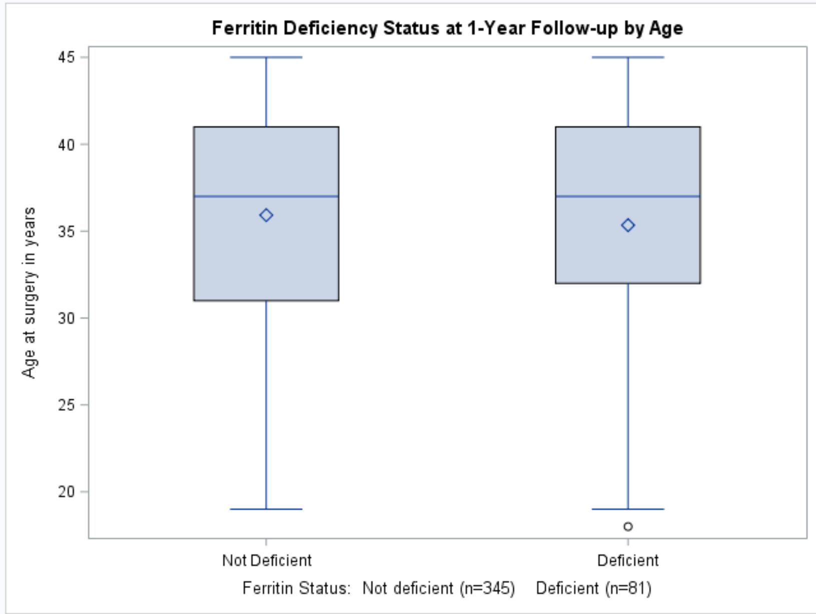
Abbreviations: LAGB: laparoscopic adjustable gastric banding

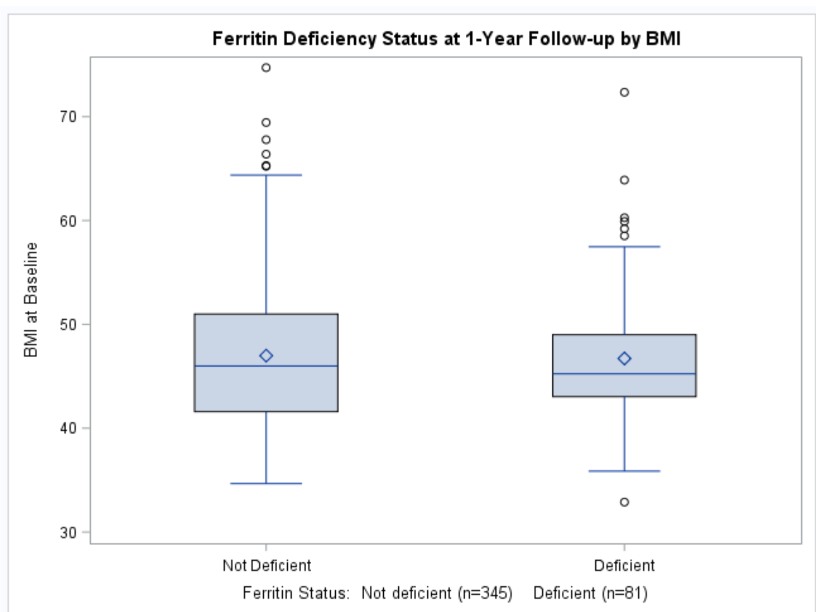
Table 29: Ferritin sufficiency status at baseline and one-year postoperative for the RYGB group

Ferritin Status at Baseline (RYGB)	Ferritin Status at 1-Year Follow-up (RYGB)			
	Deficient	Normal Range	High	Total (frequency, percent)
Deficient (frequency, row percentage)	13 68.4%	6 31.6%	0 0%	19 6.8%
Normal range (frequency, row percentage)	48 20.8%	174 75.3%	9 3.9%	231 82.5%
High (frequency, row percentage)	0 0%	14 46.7%	16 53.3%	30 10.7%
Total (frequency, percentage)	61 21.8%	194 69.3%	25 8.9%	280 100.0%

Abbreviations: RYGB: Roux-en-Y gastric bypass

Plots of individual continuous covariates versus ferritin deficiency status (not deficient or deficient) are presented in Figure 19. Contingency tables for categorical variables are presented in Tables 30 – 38.





Abbreviations: BMI: body mass index

Figure 19: Box plots of continuous variables and ferritin deficiency status

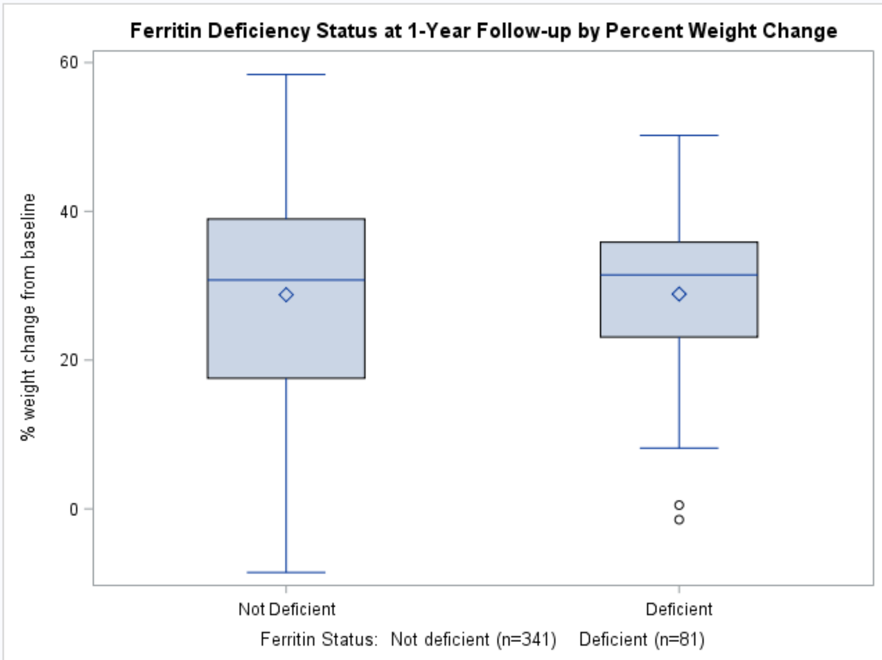


Figure 19 (continued): Box plots of continuous variables and ferritin deficiency status

Table 30: Ferritin deficiency status at one-year follow-up by type of surgery

	Deficient (n=81)	**Not Deficient** (n=345)	p
Surgery	n (%)	n (%)	0.04
LAGB (n=146)	20 (13.7)	126 (86.3)	
RYGB (n=280)	61 (21.8)	219 (78.2)	

Abbreviations: LAGB: laparoscopic adjustable gastric banding; RYGB: Roux-en-Y gastric bypass

Table 31: Ferritin deficiency status at one-year follow-up by race

	Deficient (n=81)	**Not Deficient** (n=341)	p
Race	n (%)	n (%)	0.03
White (n=365)	63 (17.3)	302 (82.7)	
Black (n=44)	15 (34.1)	29 (65.9)	
Other or Multiple Races (n=13)	3 (23.1)	10 (76.9)	
Missing (n=4)	0	4	

Table 32: Ferritin deficiency status at one-year follow-up by ethnicity

	Deficient (n=81)	**Not Deficient** (n=345)	p
Ethnicity	n (%)	n (%)	0.21
Non-Hispanic (n=393)	72 (18.3)	321 (81.7)	
Hispanic (n=33)	9 (27.3)	24 (72.7)	

Table 33: Ferritin deficiency status at one-year follow-up by annual household income

	Deficient (n=73)	**Not Deficient** (n=322)	p
Annual Income	n (%)	n (%)	0.36
<$25,000 (n=75)	16 (21.3)	59 (78.7)	
$25,000-$49,999 (n=109)	23 (21.1)	86 (78.9)	
$50,000-$74,999 (n=100)	14 (14.0)	86 (86.0)	
≥$75,000 (n=111)	20 (18.0)	91 (82.0)	
Missing (n=31)	8	23	

Table 34: Ferritin deficiency status at one-year follow-up by highest level of education completed

	Deficient (n=77)	**Not Deficient** (n=327)	p
Education	n (%)	n (%)	0.24
≤ High School (n=84)	21 (25.0)	63 (75.0)	
Some College (n=163)	28 (17.2)	135 (82.8)	
≥ College Degree (n=157)	28 (17.8)	129 (82.2)	
Missing (n=22)	4	18	

Table 35: Ferritin deficiency status at one-year follow-up by MVI with minerals supplement use at one-year follow-up

	Deficient (n=67)	**Not Deficient** (n=301)	p
MVI with Minerals Use at 1-Year Follow-up	n (%)	n (%)	0.02
None/Rarely/Monthly (n=56)	15 (26.8)	41 (73.2)	
Weekly (n=49)	12 (24.5)	37 (75.5)	
Daily (n=263)	40 (15.2)	223 (84.8)	
Missing (n=58)	14	44	

Abbreviations: MVI: multivitamin

Table 36: Ferritin deficiency status at one-year follow-up by iron supplement use at one-year follow-up

	Deficient (n=71)	**Not Deficient** (n=313)	p
Iron Supplement Use at 1-Year Follow-up	n (%)	n (%)	0.30
None/Rarely/Monthly (n=250)	50 (20.0)	200 (80.0)	
Daily or Weekly (n=134)	21 (15.7)	113 (84.3)	
Missing (n=42)	10	32	

Table 37: Ferritin deficiency status at one-year follow-up by use of histamine H_2 receptor antagonist medication at one-year follow-up

	Deficient (n=71)	**Not Deficient** (n=315)	p
Histamine H_2 Receptor Antagonist Use	n (%)	n (%)	0.30
No (n=359)	64 (17.8)	295 (82.2)	
Yes (n=27)	7 (25.9)	20 (74.1)	
Missing (n=40)	10	30	

Table 38: Ferritin deficiency status at one-year follow-up by ferritin deficiency status at baseline

	Deficient (n=81)	**Not Deficient** (n=345)	p
Ferritin Deficiency Status at Baseline	n (%)	n (%)	<.0001
Deficient (n=31)	22 (71.0)	9 (29.0)	
Not Deficient (n=395)	59 (14.9)	336 (85.1)	

Parameter estimates, p-values, adjusted odds ratios with 95% confidence intervals, and model statistics for the base multivariable logistic regression model for association of bariatric surgery and ferritin deficiency are presented in Table 39. LABS clinical site was not included in the logistic regression models because of lack of convergence due to one of the clinical sites having no cases of ferritin deficiency.

Table 39: Multivariable logistic regression for ferritin deficiency – Base Model (n=333)

P-value for the model: 0.18 **AIC**: 326.40 **c-statistic**: 0.69					
Variable	**Parameter Estimate**	**Standard Error**	**p**	**Adjusted OR**	**95% CI**
Intercept	-1.3	0.6	0.04		
RYGB (vs. LAGB)	1.5	0.5	0.01	4.56	(1.59-13.12)
Age at surgery (centered at the median)	-0.01	0.03	0.76	0.99	(0.95-1.04)
Black race (vs. white)	0.7	0.4	0.12	1.94	(0.84-4.52)
Other race or multiple races (vs. white)	0.3	0.7	0.65	1.40	(0.33-5.87)
Hispanic ethnicity (vs. non-Hispanic)	0.3	0.6	0.69	1.29	(0.38-4.37)
Some college education (vs. high school diploma or less)	-0.2	0.4	0.58	0.80	(0.36-1.77)
College degree or higher (vs. high school diploma or less)	0.02	0.4	0.97	1.02	(0.44-2.35)
Annual household income: $25,000-$49,999 (vs. <$25,000)	0.1	0.4	0.83	1.09	(0.48-2.50)
Annual household income: $50,000-$74,999 (vs. <$25,000)	-0.6	0.5	0.21	0.54	(0.20-1.43)
Annual household income: ≥$75,000 (vs. <$25,000)	-0.1	0.5	0.77	0.87	(0.34-2.21)
Baseline BMI (centered at the median)	-0.001	0.02	0.97	1.00	(0.96-1.04)
Percent weight change from baseline	-0.03	0.02	0.18	0.98	(0.94-1.01)
Weekly MVI with minerals use at the 1-year postoperative follow-up visit (vs. none/rarely/monthly use)	0.1	0.5	0.79	1.15	(0.40-3.29)
Daily MVI with minerals use at the 1-year postoperative follow-up visit (vs. none/rarely/monthly use)	-0.5	0.4	0.25	0.62	(0.27-1.40)
Daily/weekly iron supplement use at the 1-year postoperative follow-up visit (vs. none/rarely/monthly use)	-0.5	0.3	0.14	0.61	(0.32-1.18)

Abbreviations: AIC: Akaike information criterion; OR: odds ratio; CI: confidence interval; RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding; BMI: body mass index; MVI: multivitamin

The only variable that was significant in the base model was surgery. Table 40 shows the parsimonious multivariable model. Ferritin deficiency status at baseline was added to the model based its statistical significance in the simple regression model (p<.0001). When adding the other covariates stepwise to the parsimonious model, none of the variables were statistically significant. Therefore, the parsimonious model is the final model presented in Table 40. No significant interactions were identified in the final main effects model. Model diagnostics and the assumptions for multivariable logistic regression were tested for the final model. Based on the Hosmer-Lemeshow test, there was no evidence of lack of fit. There was no evidence of multicollinearity.

Table 40: Final Model: Association of bariatric surgery and ferritin deficiency based on multivariable logistic regression (n=359)

P-value for the model: <.0001 **AIC**: 311.28 **c-statistic**: 0.73					
Variable	**Parameter Estimate**	**Standard Error**	**p**	**Adjusted OR**	**95% CI**
Intercept	-2.0	0.4	<.0001		
RYGB (vs. LAGB)	1.4	0.4	0.0004	3.99	(1.85-8.59)
Black race (vs. white)	0.4	0.5	0.38	1.49	(0.61-3.64)
Weekly MVI with minerals use at the 1-year postoperative follow-up visit (vs. none/rarely/monthly use)	-0.04	0.5	0.94	0.96	(0.37-2.52)
Daily MVI with minerals use at the 1-year postoperative follow-up visit (vs. none/rarely/monthly use)	-0.9	0.4	0.02	0.40	(0.18-0.86)
Daily/weekly iron supplement use at the 1-year postoperative follow-up visit (vs. none/rarely/monthly use)	-0.5	0.3	0.12	0.59	(0.31-1.14)
Ferritin deficient at baseline (vs. not deficient)	2.7	0.5	<.0001	14.37	4.96-41.59

Abbreviations: AIC: Akaike information criterion; OR: odds ratio; CI: confidence interval; RYGB: Roux-en-Y gastric bypass; LAGB: laparoscopic adjustable gastric banding; MVI: multivitamin

Those who had undergone RYGB had greater odds of ferritin deficiency one year after bariatric surgery compared to those who had undergone LAGB, with an adjusted odds ratio (AOR) of 3.99 (95% CI: 1.85-8.59) after adjusting for the other covariates in the model (Black race, weekly MVI with minerals use at follow-up, daily MVI with minerals use at follow-up, daily/weekly iron supplement use at follow-up, and ferritin deficiency status at baseline).

4.3.4 Factors associated with change in serum ferritin level from baseline to one year after RYGB

Plots of individual covariates versus change in serum ferritin for subjects who had undergone RYGB (n=280) are presented in Figure 20 and Figure 21.

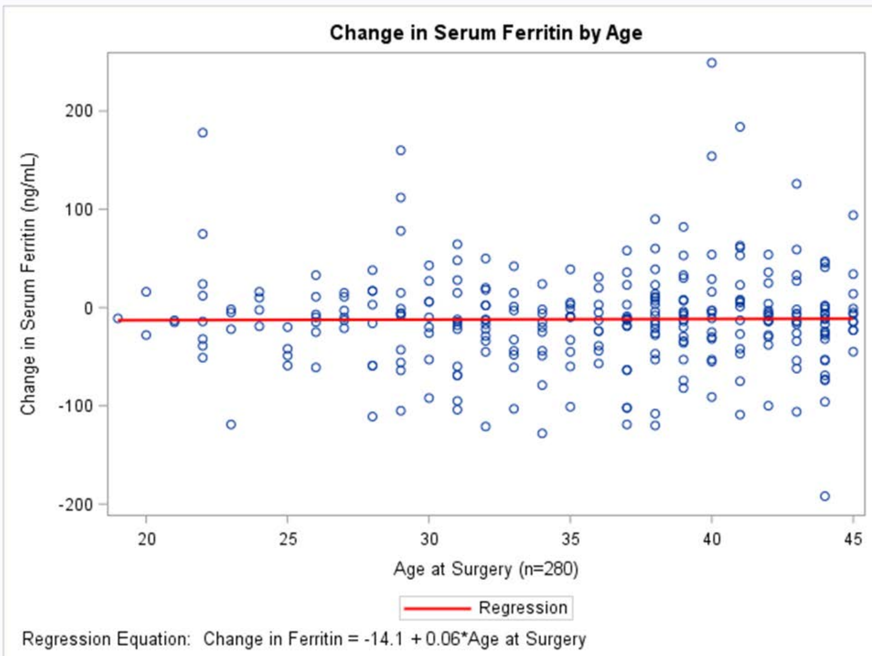
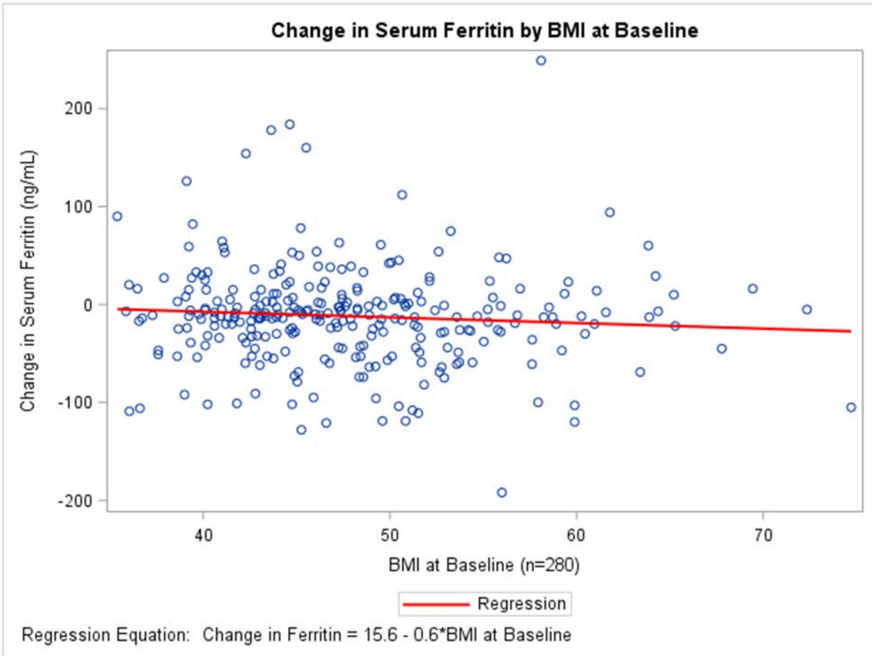


Figure 20: Scatter plots of continuous variables and change in serum ferritin after RYGB



Abbreviations: BMI: body mass index

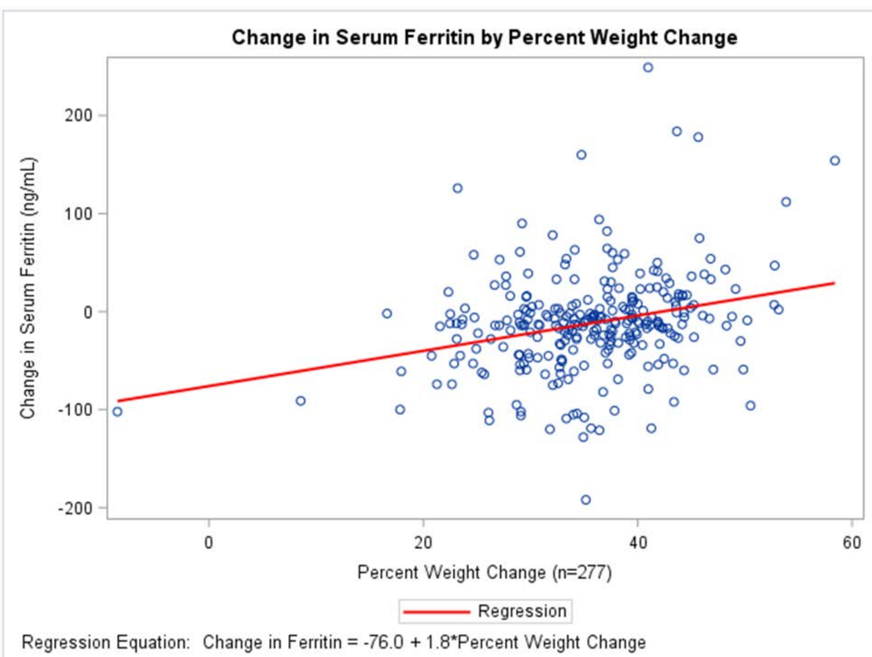
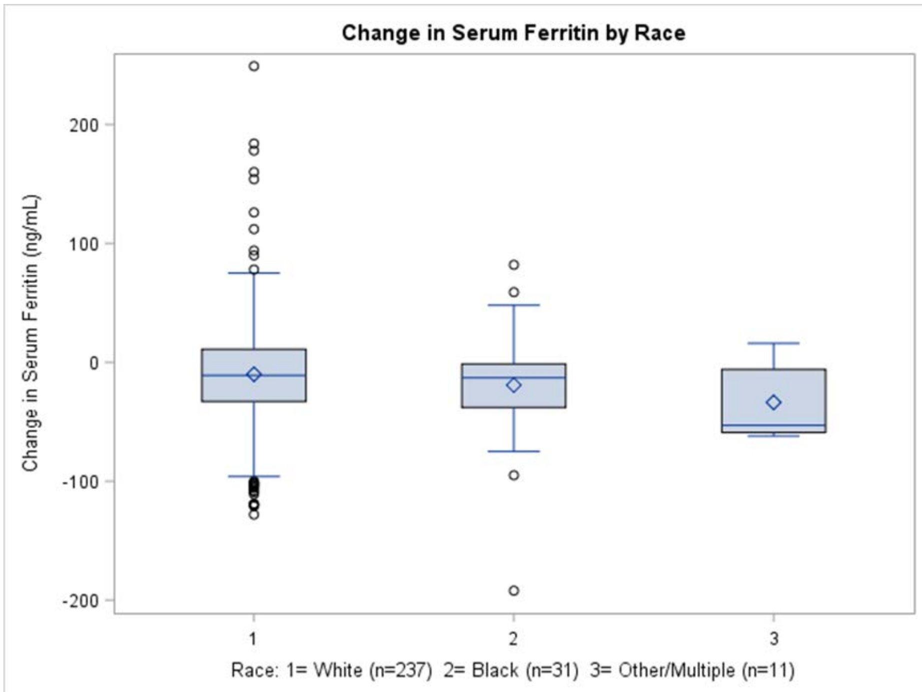


Figure 20 (continued): Scatter plots of continuous variables and change in serum ferritin after RYGB



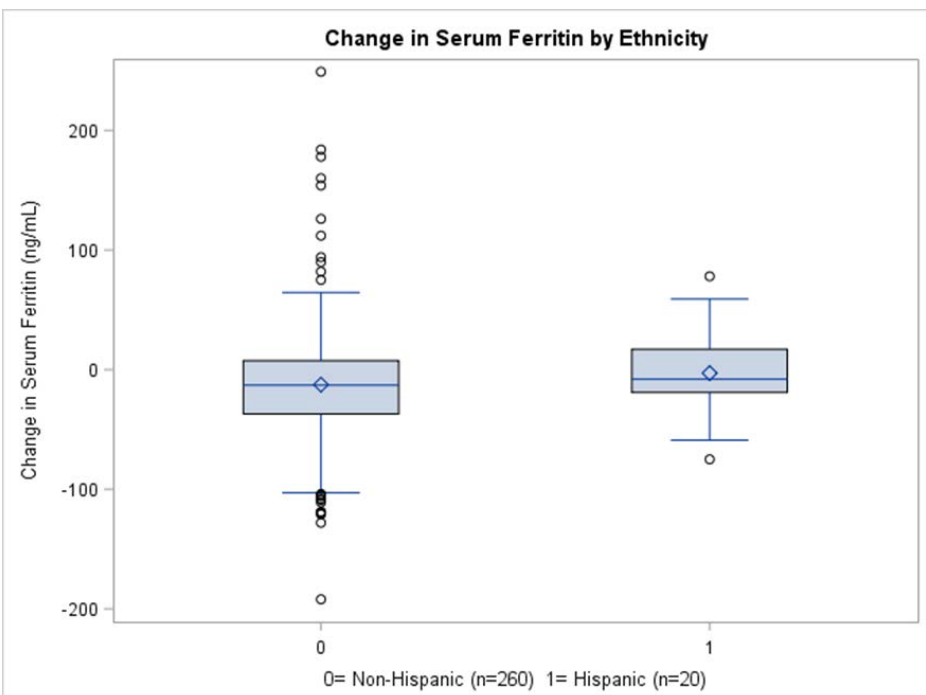


Figure 21: Box plots of categorical variables and change in serum ferritin after RYGB

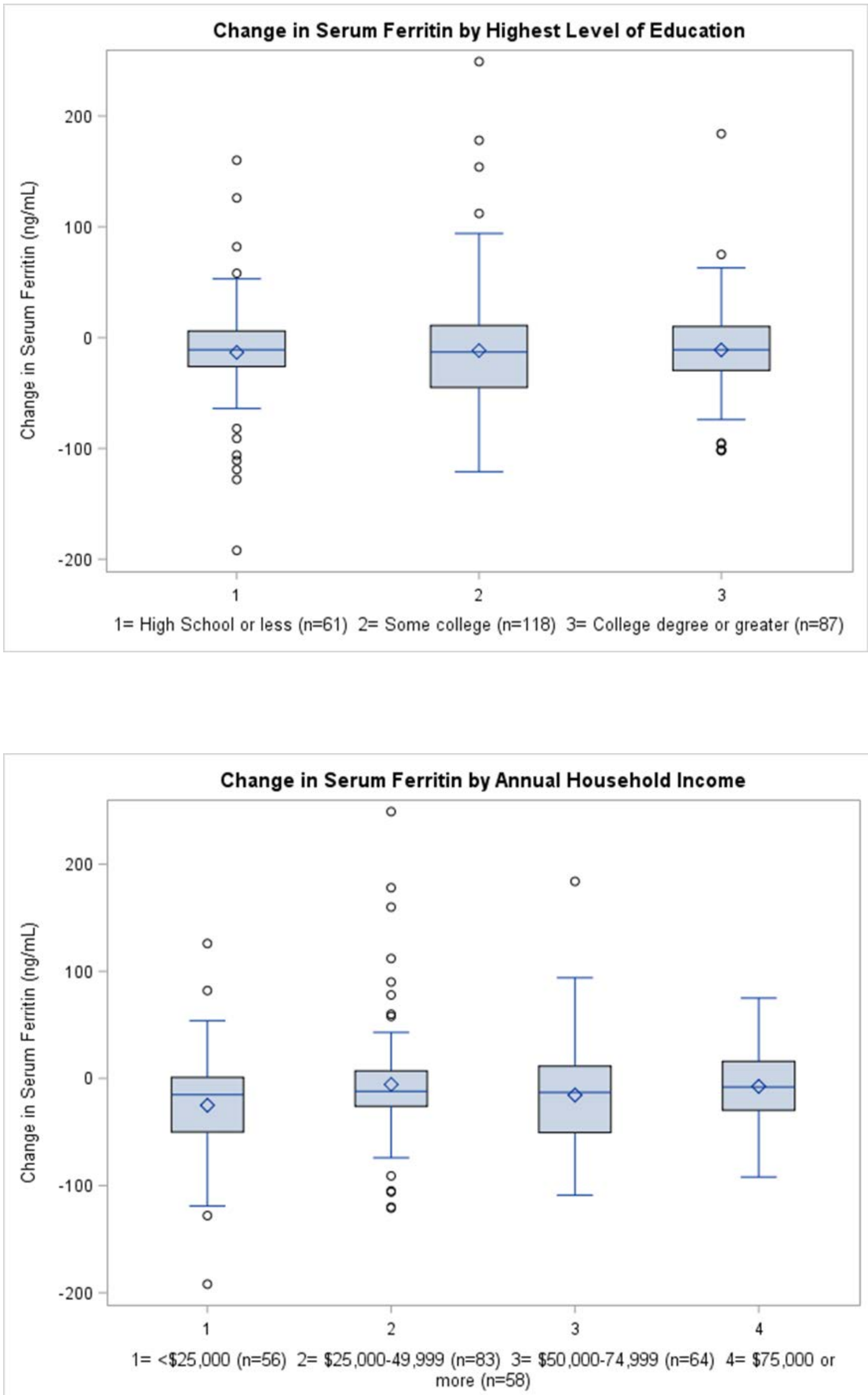
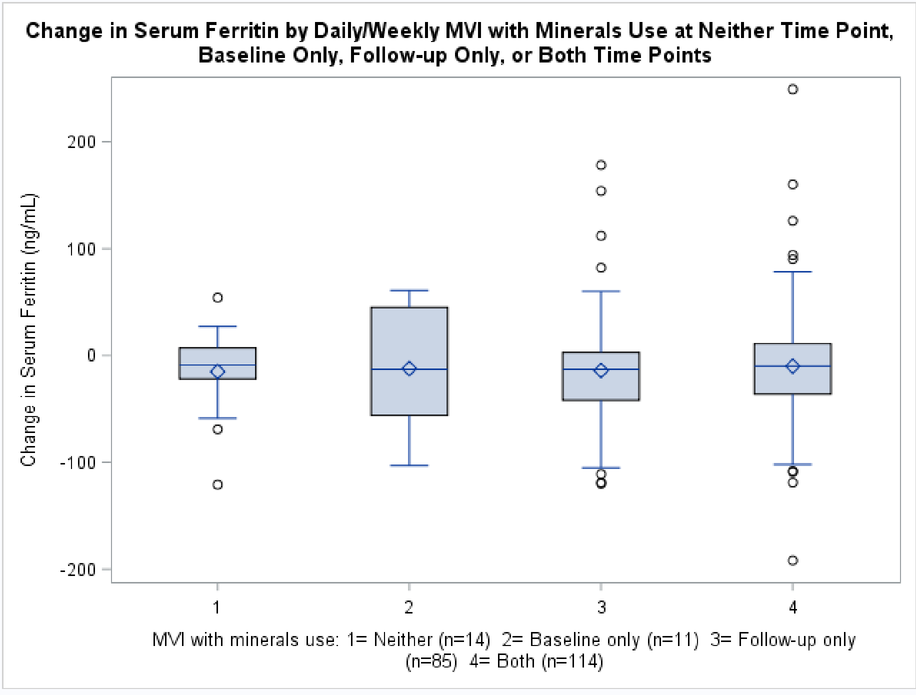


Figure 21 (continued): Box plots of categorical variables and change in serum ferritin after RYGB



Abbreviations: MVI: multivitamin

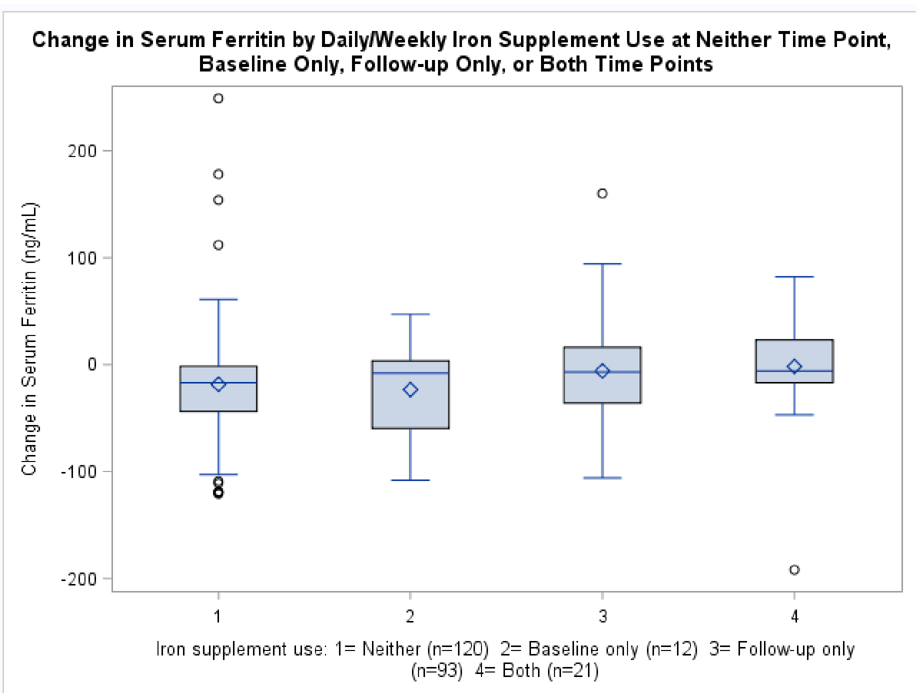


Figure 21 (continued): Box plots of categorical variables and change in serum ferritin after RYGB

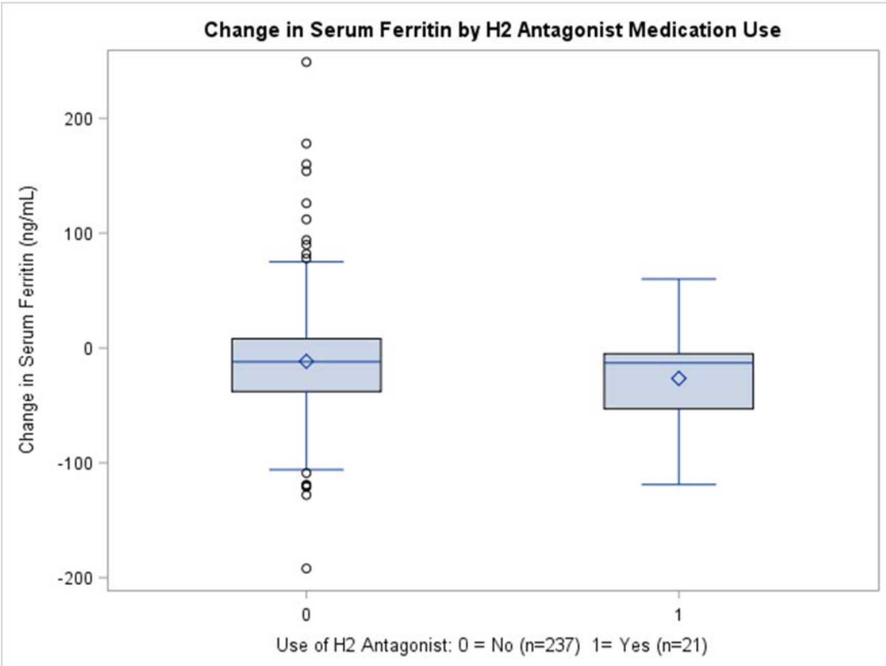


Figure 21 (continued): Box plots of categorical variables and change in serum ferritin after RYGB

Parameter estimates, p-values, and model statistics from the base multivariable model for factors associated with change in serum ferritin among women who had undergone RYGB are presented in Table 41. The reduction in the sample size for the model (compared to total n=280) is due to missing data for individual variables.

Table 41: Multivariable linear regression for change in serum ferritin after RYGB – Base Model (n=211)

P-value for the model: 0.001 **Adjusted R-squared**: 0.13			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-112.3	24.9	<.0001
Age at surgery (centered at the median)	0.4	0.6	0.47
Black race (vs. white)	-2.1	11.8	0.86
Other race or multiple races (vs. white)	-0.2	19.5	0.99
Hispanic ethnicity (vs. non-Hispanic)	2.9	16.3	0.86
Some college education (vs. high school diploma or less)	-5.7	9.4	0.54
College degree or higher (vs. high school diploma or less)	-7.9	10.4	0.45
Annual household income: $25,000-$49,999 (vs. <$25,000)	22.2	10.1	0.03
Annual household income: $50,000-$74,999 (vs. <$25,000)	1.0	11.2	0.93
Annual household income: ≥$75,000 (vs. <$25,000)	19.8	11.8	0.10
Baseline BMI (centered at the median)	-0.5	0.5	0.36
Percent weight change from baseline	2.1	0.5	<.0001
Daily/weekly MVI with minerals use at baseline only (vs. no daily/weekly use at baseline or follow-up)	20.8	21.9	0.34
Daily/weekly MVI with minerals use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	5.1	15.7	0.74
Daily/weekly MVI with minerals use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	5.4	15.5	0.73
Daily/weekly iron supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-18.3	17.4	0.29
Daily/weekly iron supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	6.9	8.7	0.43
Daily/weekly iron supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	18.0	13.4	0.18

Abbreviations: BMI: body mass index; MVI: multivitamin

Percent weight change from baseline was significant in the base model. Table 42 shows the parsimonious multivariable model. Annual household income was not included in the parsimonious model based on the results of the multiple degree of freedom test for education (p=.13).

Table 42: Multivariable linear regression for change in serum ferritin after RYGB – Parsimonious Model (n=218)

P-value for the model: <.0001 **Adjusted R-squared**: 0.14			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-109.8	22.2	<.0001
Black race (vs. white)	-5.7	11.5	0.62
Percent weight change from baseline	2.3	0.5	<.0001
Daily/weekly MVI with minerals use at baseline only (vs. no daily/weekly use at baseline or follow-up)	18.2	21.2	0.39
Daily/weekly MVI with minerals use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	4.3	15.0	0.78
Daily/weekly MVI with minerals use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	4.2	14.8	0.78
Daily/weekly iron supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-18.7	17.2	0.28
Daily/weekly iron supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	8.8	8.6	0.31
Daily/weekly iron supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	14.9	12.9	0.25

Abbreviations: MVI: multivitamin

When adding the other covariates to the parsimonious model stepwise, only baseline serum ferritin had a p<.05. Table 43 presents the final model. The variable percent weight change was modified to 5% weight change for more meaningful interpretation. No significant interactions were identified in the final main effects model. The assumptions for multivariable linear regression were tested with no violations identified. The residuals for the model were normally distributed. There was no evidence of heteroscedasticity, non-linearity between the dependent and independent variables, or collinearity among the independent variables in the final model.

Table 43: Final Model: Factors associated with change in serum ferritin level from baseline to one year following RYGB among women of childbearing age (n=218)

P-value for the model: <.0001 **Adjusted R-squared**: 0.29			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Intercept	-80.3	20.6	0.0001
Black race (vs. white)	-2.2	10.4	0.83
5% weight change from baseline	11.5	2.1	<.0001
Daily/weekly MVI with minerals use at baseline only (vs. no daily/weekly use at baseline or follow-up)	17.2	19.2	0.37
Daily/weekly MVI with minerals use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	1.8	13.6	0.89
Daily/weekly MVI with minerals use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	7.1	13.4	0.60
Daily/weekly iron supplement use at baseline only (vs. no daily/weekly use at baseline or follow-up)	-17.4	15.6	0.27
Daily/weekly iron supplement use at follow-up only (vs. no daily/weekly use at baseline or follow-up)	1.9	7.9	0.81
Daily/weekly iron supplement use at baseline and follow-up (vs. no daily/weekly use at baseline or follow-up)	7.7	11.7	0.52
Baseline serum ferritin	-0.4	0.1	<.0001

Abbreviations: MVI: multivitamin

Percent weight change from baseline was significantly (p<.0001) associated with change in serum ferritin among women who underwent RYGB. For every 5% weight change from baseline, the change in serum ferritin increases by 11.5 ng/mL, after adjusting for the other covariates in the model (Black race, daily/weekly MVI with minerals use, daily/weekly iron supplement use, and baseline serum ferritin), and the fixed effects of LABS clinical site. The overall clinical site effect was significant (p=.01) so clinical sites were retained in the final model. This model confirms that the association between percent weight change and serum ferritin differs by type of surgery. Baseline serum ferritin level was also significantly (p<.0001) associated with change in serum ferritin, after adjusting for the other covariates in the model and the fixed effects of LABS clinical site.

5.0 DISCUSSION

The primary purpose of this research was to determine the association between two types of bariatric surgery, RYGB and LAGB, and the change in serum folate and change in serum ferritin from prior to surgery to one year following surgery among women of childbearing age (18-45 years). The hypothesis for this aim was that women who underwent RYGB would have a larger decrease in serum folate and serum ferritin compared to women who underwent LAGB due to issues related to malabsorption of micronutrients. This research also aimed to determine the prevalence of folate and iron deficiency prior to and one year after RYGB and LAGB and to identify factors associated with change in serum folate and change in serum ferritin levels specifically among women of childbearing age who had undergone RYGB.

The population of interest for the study was women of childbearing age due to the important role of folate and iron during pregnancy for proper fetal development. It is recommended that women avoid becoming pregnant for 12-18 months following bariatric surgery due to the increased risk of micronutrient deficiencies associated with bariatric surgery.[11] The study had 413 subjects (272 RYGB, 141 LABG) for the folate analyses and 426 subjects (280 RYGB, 146 LAGB) for the serum ferritin analyses. The subjects were a subset of the female participants from the LABS-2 study.

To investigate the aims of the study, serum samples stored at the NIDDK Central Repository were used to determine preoperative and one-year postoperative serum folate and serum ferritin levels for each subject. Serum ferritin was used as an indicator of iron status. The time point of one year following surgery was selected because it falls at the beginning of the time period when it is considered safe for women to become pregnant after bariatric surgery. The serum analyses were conducted by the staff at the Heinz Nutrition Laboratory. The change in serum folate and change in serum ferritin from baseline to one year following bariatric surgery as well as deficiency status for each micronutrient were determined and statistical analyses were conducted to address the second and third aims of the study.

5.1 CHANGE IN SERUM FOLATE AND FOLATE DEFICIENCY AFTER RYGB AND LAGB

5.1.1 Change in serum folate from baseline to one year following bariatric surgery among women of childbearing age

The results of the folate analyses presented a number of unexpected findings including median values for serum folate at one year after surgery within the normal range for the women who underwent LAGB (22.3 ng/mL) and above the normal range for the women underwent RYGB (26.0 ng/mL). Blume et al.[89], Donadelli et al.[91], Coupaye et al.[94], and Coupaye et al.[95] reported increases in the median or mean serum folate levels from pre-op to one year after RYGB; however, the median or mean serum levels at one year post-op were not as high as in this study (range of 13.3–17.6 ng/mL). The mean change in serum folate after LAGB was a decrease of 4.3 ng/mL from baseline to one year after surgery, and the mean change after RYGB was an increase of 2.8 ng/mL.

Based on multivariable linear regression, there was a not a significant difference in the change in serum folate from baseline to one year after surgery by type of surgery, after adjusting for other covariates in the model. These results are in contrast to the hypothesis for the study that women who underwent RYGB would have a greater decrease in serum folate from baseline to one year after surgery than women who underwent LAGB.

When focusing on the women who underwent RYGB (n=272) to address the third aim of the study, daily/weekly MVI use at follow-up only and daily/weekly MVI use at both baseline and follow-up were negative confounders of the relationship between RYGB and change in serum folate. Daily/weekly MVI use at follow-up only was associated, on average, with a 10.9 ng/mL greater change in serum folate (p=.04), and daily/weekly MVI use at both baseline and follow-up was associated, on average, with a 12.3 ng/mL greater change in serum folate (p=.02) compared to no daily/weekly use at either time point, after adjusting for the other covariates in the model. Surprisingly, daily/weekly use of a folate supplement at both baseline and follow-up, on average, resulted in a 17.5 ng/mL lower change in serum folate level (p=.04) compared to no daily/weekly use of a folate supplement at baseline or follow-up, after adjusting for the other covariates in the model. As would be expected, baseline serum folate was significantly (p<.0001) associated with change in serum folate, after adjusting for the other covariates.

5.1.2 Bariatric surgery and the prevalence of folate deficiency

The prevalence of folate deficiency for all subjects (n=413) was low before surgery (0.2%) and one year after surgery (1.0%) and did not differ significantly by type of surgery (0.7% after LAGB vs. 1.1% after RYGB; p=1.00). The prevalence of folate deficiency one year after surgery was similar to the overall prevalence of 0.9% among women of childbearing age in the

U.S.[61] At one year after LAGB, the prevalence of folate deficiency found in this study was lower than the 10.0% prevalence of deficiency (based on serum folate equivalent to <3 ng/mL) reported by Coupaye et al.[94] in a small study of 21 subjects (18 women, 3 men). The prevalence of deficiency one year after RYGB was similar to what was reported by Blume et al.[89], Donadelli et al.[91], Coupaye et al.[94], and Coupaye et al.[95] but lower than what was reported by Dogan et al.[90] and Toh et al.[93] one year after surgery. Blume et al.[89] reported 1.2% prevalence of deficiency based on serum folate <2.8 ng/mL in a study of 170 subjects (136 women, 34 men). In a study of 58 subjects (46 women, 12 men), Donadelli et al.[91] reported 3.4% prevalence of deficiency based on serum folate <3 ng/mL. Coupaye et al.[94] reported no cases of folate deficiency one year after RYGB using diagnostic criteria of serum folate equivalent to <3 ng/mL in a cohort of 49 subjects (45 women, 4 men). In their 2014 study, Coupaye et al.[95] reported 3.0% prevalence of folate deficiency among 43 subjects (31 women, 12 men) using the same diagnostic criteria as in their prior study. In contrast, Dogan et al.[90] reported 6.8% prevalence of deficiency in a cohort of 74 subjects (51 women, 23 men) based on serum folate equivalent to <3.97 ng/mL. Toh et al.[93] reported 12.0% prevalence of folate deficiency in a study of 103 subjects (number of women vs. men not specified); however, RBC folate was used to identify deficiency rather than serum folate. Similar to this study, the majority of researchers used serum folate to define folate deficiency with a range of <2.8-4.0 ng/mL.

One year after surgery, the majority of the women in this study who had undergone LAGB had serum folate levels within the normal range (55.3%) or above the normal range (44.0%). For those who underwent RYGB, 46.0% had serum folate levels within the normal range and 52.9% had levels above the normal range one year following surgery.

The use of MVI supplements (which typically contain folate) may have played a role in these findings since the percentage of women who reported daily MVI supplement use at the one-year postoperative visit was 58.0% for women who underwent LAGB and 83.8% for those who underwent RYGB. The American Society for Metabolic and Bariatric Surgery recommends that women of childbearing age take 800-1000 µg per day of supplemental folate from their multivitamin.[23,28] MVI supplements commonly provide 400 µg in one dose[49], but amounts provided in supplements can vary widely. Due to the potential to mask vitamin B_{12} deficiency, supplementation of folate above 1000 µg per day is not recommended.[23] The serum folate test used in this study is an indicator of short-term folate status and recent intake rather than long-term folate status.[51,55] Therefore, the serum levels may have been elevated due to recent supplement use.

Another possible cause of the high serum folate levels is vitamin B_{12} deficiency[55], a commonly reported deficiency related to bariatric surgery[10]. However, determination of vitamin B_{12} status was beyond the scope of this study.

Based on the results of this study, folate deficiency at one year after LAGB and RYGB was rare. However, these results should be interpreted with caution based on the diagnostic test used to identify folate deficiency, as discussed in the Study Limitations section.

A potential concern, based on the results of the study, is the large proportion of subjects with serum folate levels above the normal range. It may be possible that individuals were receiving too much folic acid after bariatric surgery from a combination of supplements containing folic acid and consumption of grains and cereals fortified with folic acid. Consequences of excessive folate supplementation and resulting high serum folate levels must be considered. A recent study by Raghavan et al.[110] of 1257 mother-child pairs from Boston

Medical Center reported that mothers with plasma folate levels of ≥ 60.3 nmol/L at birth (equivalent to ≥ 26.6 ng/mL) had a higher risk of having a child with Autism Spectrum Disorder [adjusted hazard ratio (HR) of 2.5; 95% CI: 1.3-4.6]. Mothers who reported taking a MVI supplement more than five times a week during pregnancy also had a higher risk of having a child with Autism Spectrum Disorder [adjusted HR of 2.3 (95% CI: 1.2-3.9) for MVI use in the first trimester; adjusted HR of 2.1 (95% CI: 1.2-3.6) for MVI use in the second and third trimesters].

5.2 CHANGE IN SERUM FERRITIN AND IRON DEFICIENCY AFTER RYGB AND LAGB

5.2.1 Change in serum ferritin from baseline to one year following bariatric surgery among women of childbearing age

The unadjusted mean change in serum ferritin was a decrease of 3.8 ng/mL after LAGB and a decrease of 11.9 ng/mL after RYGB. In the multivariable linear regression model, there were significant interactions between type of surgery with both percent weight change from baseline and baseline serum ferritin level, such that the difference in change in serum ferritin between RYGB and LAGB was larger with less weight loss and higher baseline ferritin level.

These interactions also showed that the associations of percent weight change and baseline ferritin, respectively, with change in serum ferritin were more pronounced among women who had undergone RYGB compared to LAGB.

The third aim of the study was to determine factors associated with change in serum ferritin specifically among women of childbearing age who underwent RYGB (n=280). Percent weight change from baseline was significantly ($p<.0001$) associated with change in serum ferritin from baseline to one year after RYGB. For every 5% weight change from baseline, serum ferritin increased by 11.5 ng/mL, after adjusting for the other covariates in the model and the fixed effects of clinical site. Baseline serum ferritin was significantly ($p<.0001$) associated with change in serum ferritin, after adjusting for the other covariates and fixed effects of clinical site.

The direction of the relationship between percent weight change and change in serum ferritin was in contrast with the hypothesis that a greater weight loss would be associated with a greater decrease in serum ferritin since the mechanisms that produce weight loss after bariatric surgery (i.e. decreased oral intake and malabsorption) could also increase the risk of nutrient deficiencies.[23] However, it has been suggested that weight loss could play a role in increasing serum ferritin levels.[111] Ferritin can be stored in activated hepatic adipocytes.[112] A possible mechanism of action for higher serum ferritin levels with greater weight loss could be increased release of ferritin due to hepatic lipolysis to meet energy needs. Also, the inflammation associated with obesity impairs intestinal absorption of iron, and so, weight loss may improve intestinal iron absorption.[113]

MVI with minerals supplement use (which often contain iron) and iron supplement use were not found to have a significant association with change in serum ferritin after RYGB. It is possible that there may have been a lack of power to detect an association due to missing data for these variables. Twenty percent of the data for the daily/weekly MVI with minerals supplement use variable and 12.1% of the data for the daily/weekly iron supplement use variable were missing for the subjects who underwent RYGB. The parameter estimates for these variables were in the expected direction of greater change in serum ferritin with supplement use and had wide 95% confidence intervals, which indicate that lack of power may have been an issue. Black race was also not significantly associated with change in serum ferritin. Due to the low prevalence of African Americans in the study (10.4% of the total subjects), there may have been inadequate power to detect a significant association.

5.2.2 Bariatric surgery and the prevalence of iron deficiency

Prior to surgery, 7.3% of the women in the study had iron deficiency, as determined by serum ferritin level, as compared to the overall prevalence for women aged 20-49 years in the U.S. of 13.2%.[79] One year following surgery, the prevalence of iron deficiency for the total sample (n=426) increased significantly ($p<.0001$) to 19.0%. The prevalence of iron deficiency was significantly ($p=0.04$) greater among women who had undergone RYGB than LAGB (21.8% vs. 13.7%, respectively). In the unadjusted analysis, the prevalence of iron deficiency was 34.1% for African American women vs. 17.3% for Caucasian women. Based on multivariable logistic regression, women who had undergone RYGB had significantly greater odds of iron deficiency one year after surgery compared to women who had undergone LAGB (AOR 3.99; 95% CI: 1.85-8.59), after adjusting for the covariates in the model.

The prevalence of iron deficiency one year after LABG was higher than reported by Coupaye et al.[94] (5.0%) in a study of 21 subjects (18 women, 3 men). However, Coupaye et al.[94] used a stricter diagnostic criterion for iron deficiency of serum ferritin equivalent to <3 ng/mL which would lead to fewer subjects being classified as iron-deficient.

For RYGB, the prevalence of iron deficiency was higher than what was reported in the studies by Bavaresco et al.[87], Blume et al.[89], Dogan et al.[90], Ikramuddin et al.[92], Toh et al.[93], and Coupaye et al.[94] at one year after surgery. Bavaresco et al.[87] reported 14.6% prevalence of iron deficiency in a cohort of 48 subjects (41 women, 7 men) but used serum iron, rather than serum ferritin, as the diagnostic test. In a study of 170 subjects (136 women, 34 men), Blume et al.[89] reported 4.1% prevalence of deficiency based on serum ferritin <10 ng/mL. Dogan et al.[90] reported 10.7% prevalence of iron deficiency in a cohort of 74 subjects (51 women, 23 men) based on diagnostic criteria of serum ferritin equivalent to < 20 ng/mL. Ikramuddin et al.[92] reported 14.0% prevalence of iron deficiency among 60 subjects (38 women, 22 men); however, diagnostic criteria for defining deficiency was not reported. In a study of 103 subjects (number of women vs. men not specified), Toh et al.[93] reported 15.0% prevalence of iron deficiency using the same diagnostic criteria as this study. In the 2009 study by Coupaye et al.[94], the prevalence of deficiency was 4.0% among a cohort of 49 subjects (45 women, 4 men) using a much stricter diagnostic criterion of serum ferritin equivalent <3 ng/ml. The prevalence of iron deficiency one year after RYGB was similar to the 20% prevalence reported in the 2014 study by Coupaye et al.[95] of 43 subjects (31 women, 12 men); however, the researchers used transferrin saturation rather than serum ferritin to identify deficiencies. Differences in criteria used to identify iron deficiency may account for some of the differences in reported prevalence.

The results of this study indicate that iron deficiency is a concern for women at one year after LAGB and RYGB, but particularly for women after RYGB. The prevalence of iron deficiency one year after LAGB is similar to the overall prevalence for women aged 20-49 years in the U.S., but the prevalence after RYGB is higher. Based on the results of this study, it is recommended that all women be tested for iron deficiency at one year after RYGB using a diagnostic test that is an indicator of early-stage iron deficiency such as serum ferritin and then treated if deficient to avoid the risks associated with iron deficiency in the periconceptional period and during pregnancy (e.g. maternal iron deficiency anemia during pregnancy, low infant iron stores at birth[70], greater risk of preterm birth[71-73], greater risk of infant low birth weight[71], and interference with fetal neurodevelopment leading to difficulties with learning, language skills, emotional development, and motor skills[74,75]) and the consequences of iron deficiency for all adults (e.g. iron deficiency anemia, epithelial tissue disorders, decreased muscle function and exercise tolerance, and fatigue[69]).

5.2.3 Pregnancy status

Pregnancy was not an exclusion criterion for the study. Therefore, pregnant and possibly pregnant women were included in all analyses. There were four women (0.9% of subjects) who reported being pregnant and nine women (2.1% of subjects) who reported possibly being pregnant at the one-year postoperative follow-up visit. The multivariable regression analyses for change in serum folate, change in serum ferritin, and ferritin deficiency status were conducted with and without the four women who reported being pregnant, with no significant differences in the results. Therefore, the data for the pregnant women were included in the results of all statistical analyses.

For the women who reported being pregnant, none were deficient in folate at baseline or at the one-year postoperative follow-up visit. For iron deficiency, based on serum ferritin, none were deficient at baseline and one woman was deficient at the one-year postoperative follow-up visit. For supplement use, two of the women reported taking a MVI with minerals daily at the one-year postoperative follow-up visit (data was missing for the other two women). For folate and iron supplement use, data was missing for one subject. Of the other three women, none were taking a folate supplement, and one women reported taking an iron supplement on a daily basis at follow-up.

5.3 STUDY LIMITATIONS

The study had some limitations that should be noted. LAGB is no longer as commonly performed as it was during the years when the LABS participants were undergoing surgery, and it has largely been replaced by the sleeve gastrectomy.[1] The results from this study related to LAGB may not be as relevant at the current time.

Self-report data were used for many of the covariates (i.e. supplement use, alcohol use) that were investigated for association with the outcomes of the study. Results dependent on self-report data can be subject to social desirability and recall bias. Also, after bariatric surgery, as individuals may be able to decrease the dose or discontinue some of their medications due to improvements in obesity-related comorbidities[114], there may be some hesitancy to take other medications or supplements.

In addition, there were missing data for a number of the self-report variables. In particular, MVI with minerals supplement use at follow-up (13.6% missing), number of alcoholic drinks consumed per day at follow-up (12.7%), alcohol use disorder symptoms at follow-up (12.2%), frequency of alcohol consumption at follow-up (12.0%), MVI with minerals use at baseline (10.6%), and iron supplement use at follow-up (9.9%). Missing data were more prevalent at the one-year postoperative follow-up visit than at baseline.

While frequency of use data were available for the micronutrient supplements (MVI, MVI with minerals, folate, and iron), dosage was not available. Brand names for the MVI and MVI with mineral supplements, which could have been used to determine dosage, were also not available. The American Society for Metabolic and Bariatric Surgery recommends that individuals take a MVI with minerals supplement that provides 100% of the daily value for at least two-thirds of the nutrients after LAGB and a MVI with minerals supplement that provides 200% of the daily value for at least two-thirds of the nutrients after RYGB (e.g. taking two MVI with minerals supplements daily).[23] However, the dosage of supplements can vary greatly which could affect serum levels of the nutrient. MVI and MVI with minerals supplements can range from providing very little or no dosage of a nutrient to providing an amount that exceeds the DRI's Tolerable Upper Intake Levels. Table 44 provides examples of MVI with minerals supplements and the variation in folic acid and iron provided.

Table 44: Folic acid and iron content of various MVI with minerals supplements

Brand Name of Supplement (Manufacturer directions and serving size)	Folic Acid	Iron
Centrum Chewables, Orange Burst[115] (1 tablet, once daily)	400 µg	8 mg
Bariatric Advantage Ultra Multi with Iron[116] (3 capsules, once daily)	800 µg	45 mg
BariActiv Multivitamin Chewable[117] (2 tablets, once daily)	800 µg	0 mg
One A Day Women's Prenatal[118] (1 tablet, once daily)	800 µg	28 mg
Nature's Plus Exotic Red Super Fruits Adult's Multi-Vitamin Chewable[119] (1 tablet, once daily)	100 µg	5 mg

Another limitation to the study was poor model fit for the multivariable linear regression models for change in serum folate and change in serum ferritin. The adjusted R-squared values are presented in Table 45. It is possible that undetected confounding (e.g. no data available on dietary intake) may have biased the regression results.

Table 45: Adjusted R-squared values for the multivariable linear regression models

Model	Adjusted R-squared
Association of bariatric surgery and change in serum folate	0.28
Factors associated with change in serum folate after RYGB	0.24
Association of bariatric surgery and change in serum ferritin	0.23
Factors associated with change in serum ferritin after RYGB	0.29

Finally, for practical reasons, serum folate was used to determine folate status rather than RBC folate concentration. Serum folate level is an indicator of short-term folate status and is affected by recent dietary intake.[51,55] The RBC folate test is an indicator of longer-term tissue folate status.[51-53] It is likely that the use of MVI supplements containing folic acid and folate supplements increased the serum folate values, and perhaps, was responsible for the large proportion of subjects having serum levels above the normal range. It is also possible that there may have been cases of folate deficiency that were not identified due to elevated serum folate levels based on recent dietary or supplemental intake of folate/folic acid.

5.4 STUDY STRENGTHS

The study had a number of strengths.   This study was conducted as an ancillary study to the LABS study which was a multicenter study. Subjects for the study were selected from ten clinical centers in different geographic areas of the U.S., which increased the generalizability of the study. The researchers had access to a large amount of data that had been collected by LABS-trained personnel which were used as variables for the study. Descriptive data on these variables were reported, adding to the body of knowledge produced from the LABS studies (i.e. prevalence of folate and iron deficiencies among women of childbearing age and reported micronutrient supplement use). The study also had a larger sample size (n=413 for the folate analyses and n=426 for the ferritin analyses) compared to prior studies discussed in the literature review. Unlike many of the prior studies, the current study examined the association of bariatric surgery and a number of covariates on the change in serum folate and change in serum ferritin. Finally, there was good model fit (c-statistic: 0.73) for the multivariable logistic regression model which determined the association of bariatric surgery and ferritin deficiency.

5.5 PUBLIC HEALTH SIGNIFICANCE

This research is relevant to public health because of the need for proper nutrition in pregnant women. This study provides evidence that bariatric surgery, RYGB in particular, presents a potential risk to perinatal health due to iron deficiency for women who become pregnant at one year after surgery. One year after RYGB, 21.8% of women were deficient in iron. Maternal anemia during pregnancy is associated with a greater risk of preterm birth[71-73], low birth weight for the infant[71], and difficulties with learning, language skills, emotional development, and motor skills during childhood[74,75]. The results of this research contribute to the body of knowledge related to the Healthy People 2020 goal to "improve the health and well-being of women, infants, children, and families".[120] In the U.S., 9.6% of infants are born preterm and 8.1% are born with low birth weight.[121] Based on the results of this research, all women should be tested for iron deficiency one year after RYGB. This is in agreement with the American Society for Metabolic and Bariatric Surgery recommendation that starting at twelve months after bariatric surgery iron status should be monitored annually.[28] The *Committee on Obstetric Practice of the American College of Obstetricians and Gynecologists* also recommends that women who have undergone bariatric surgery and are pregnant, or are planning to become pregnant, should be evaluated for potential iron deficiency.[40] There is evidence to support delaying pregnancy by at least two years after bariatric surgery. Parent et al.[12] reported higher risk of infant prematurity, neonatal intensive care admission, and small for gestational age status for women who gave birth less than two years after surgery compared to women who gave birth greater than four years after bariatric surgery.

5.6 DIRECTIONS FOR FUTURE RESEARCH

Based on the outcomes and limitations of this study, a number of recommendations can be made for future research directions. The first recommendation would be to conduct future studies using RBC folate concentration as the diagnostic test for folate status rather than serum folate since RBC folate test is an indicator of longer-term tissue folate status and would be less affected by recent intake of supplements containing folate and dietary intake.[51-53]. Second, continued investigation of the prevalence of iron deficiency after RYGB is suggested including time points farther out from surgery to determine if iron deficiency continues to be a concern at 18 months, two years, etc. These data would be valuable to determine if the current recommendations for the duration of time women should wait to become pregnant after RYGB should be revised.

In addition, it is advisable that future researchers collect more detailed data on micronutrient supplement usage including the dosage of folate and iron in individual supplements and MVI or MVI with minerals supplements, how often supplements are taken in a day, and the brand name of supplements. Due to the low percentage of variance explained by the multivariable linear regression models investigating the association of bariatric surgery with changes in serum ferritin and folate, respectively, researchers should consider other factors not included in the study that may have an association such as dietary intake. Finally, a similar study should be conducted with women of childbearing age who have undergone sleeve gastrectomy, which is currently performed more commonly than LAGB[1].

5.7 CONCLUSION

The primary goals of this study were to: 1) investigate the association of two types of bariatric surgery, LAGB and RYGB, with change in serum folate and change in serum ferritin from prior to surgery to one year following surgery among women of childbearing age; 2) determine factors associated with change in serum folate and change in serum ferritin after RYGB; and 3) determine the prevalence of folate and iron deficiency one year after surgery. The results showed that type of surgery was not significantly associated with change in serum folate, after adjusting for other covariates, but this may have been a function of the limitations in the assay which reflects short-term folate status and can be affected by recent intake. Hence, additional research is recommended using RBC folate concentration instead of serum folate to determine folate status. The results of this research indicate that, despite the positive effect of multivitamin with minerals supplement use, iron deficiency is a concern at one year after RYGB and monitoring for iron deficiency one year after surgery is recommended for all women.

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