CHANGES IN SERUM HOMOCYSTEINE LEVELS AFTER ROUX-EN-Y GASTRIC BYPASS SURGERY IN SEVERE OBESITY

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DEDICATION

To Mom and Dad-
For raising me and putting me in the position to be here and write this. Without your ceaseless prayers, support, and love through the years, I would probably be selling hot dogs on the corner, or quite possibly in jail. Thank you for all that you have done and continue to do for me/us.

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ABSTRACT

Homocysteine is a sulfur-containing amino acid that is formed after the demethylation of methionine. High levels of plasma homocysteine have been proposed as a major and independent risk factor for cardiovascular disease. It is well known that obesity is also a modifiable and independent risk factor for cardiovascular disease. Extensive research has shown that weight loss produced by both traditional methods (dietary restriction/exercise) and gastric bypass surgery can improve several well known cardiovascular risk factors. As the rates of morbid obesity have been rapidly growing, the number of gastric bypass surgeries has also drastically increased, with Roux-en-Y gastric bypass (RNYGBP) surgery being the most common. While it is relatively well known that gastric bypass and weight loss improve many other risk factors for cardiovascular disease, the literature has been very limited regarding the effect of gastric bypass surgery on plasma homocysteine concentrations. The purpose of this study is to examine the effect of this surgery on the emerging cardiovascular disease risk factor of high plasma homocysteine. Participants were recruited from patients scheduled to undergo gastric bypass surgery at the Wake Forest University Baptist Medical Center General Surgery Clinic. Men and women were eligible for the study if they had a BMI $\geq$ 40.0 kg/m^2 or $\geq$ 35.0 kg/m^2 with an obesity related comorbidity, such as hypertension, dyslipidemia, or diabetes. Participants (n=19 female; age=45.7 ($\pm$8.8) years; BMI=54.4 ($\pm$7.2) kg/m^2 had blood drawn prior to, and at 3 weeks, 3 months, 6 months, and 12 months post-surgery. Blood serum was analyzed for homocysteine, as well as vitamins B-12 and folate because these water-soluble B vitamins are determinants of serum homocysteine levels. One-way repeated measure ANOVA determined effect of the surgery on homocysteine over time.

Weight loss was -7.8 (±1.5)% at 3 weeks, -17.7 (±2.7)% at 3 months, -26.0 (±4.0)% at 6 months, and -33.8 (±7.1)% at 12 months. Serum folate and vitamin B-12 did not significantly change from baseline to 12 months. Serum homocysteine levels were 10.5 (±3.6) μM/L at baseline, 11.8(±3.9) μM/L at 3 weeks, 11.4(±3.9) μM/L at 3 months, 11.7(±2.9) μM/L at 6 months, and 10.4 (±2.4) μM/L at 12 months. Serum homocysteine levels did not significantly change during the study. At baseline and throughout the course of the study, 95% of the participants had normal homocysteine levels. Change in homocysteine at twelve months was significantly negatively correlated to change in BMI as well as change in weight between baseline values and other visits. Results indicate that when vitamin status is controlled, Roux-en-Y gastric bypass surgery will not significantly alter homocysteine levels in morbidly obese patients.

LITERATURE REVIEW

INTRODUCTION

Homocysteine is a sulfur-containing amino acid that is formed after the demethylation of methionine.[99, 100, 138] High levels of plasma homocysteine have been proposed as a major and independent risk factor for cardiovascular disease.[54, 99-101, 135, 138] It is well known that obesity is also a modifiable and independent risk factor for cardiovascular disease[169]. Extensive research has shown that mass loss produced by both traditional methods (dietary restriction/exercise) [6, 95, 98] and gastric bypass surgery [94, 168, 217] can improve several well known cardiovascular risk factors. As the rates of morbid obesity have been rapidly growing, the use of gastric bypass surgeries to promote mass loss has also drastically increased, with Roux-en-Y gastric bypass (RNYGB) surgery being the most common.[5, 48, 63, 180] While it is relatively well known that gastric bypass and mass loss improve many other risk factors for cardiovascular disease, the literature has been very limited regarding the effect of gastric bypass surgery on plasma homocysteine concentrations.[24, 53, 94, 99, 118] Thus, it is of interest to examine if this surgery alters levels of the emerging cardiovascular disease risk factor of high plasma homocysteine.

OBESITY

Definition

Obesity is commonly defined as an abnormal or excessive amount of body fat that leads to impaired health.[204] While there are many ways to establish the presence of obesity, the most commonly used definition is body mass index (BMI). BMI is the ratio of mass (kg) to height squared (m^2). Typically, a high BMI will indicate a high amount of body fat, as well as increased health risks.[79] While the level of adiposity shows a high inter-individual variation for a given BMI, overall BMI has been shown to be a relatively accurate predictor of adiposity on the population level.[19, 79] A person with a BMI over 30 kg/m^2 is considered obese. There are three classes of obesity. Class 1 obesity is a BMI between 30.0 and 34.9 kg/m^2; class 2 is between 35.0 kg/m^2 and 39.9 kg/m^2; and class 3 is greater than 40.0 kg/m^2. Class 3 obesity is often referred to as "Morbid Obesity".[2] In recent years, increased attention has been paid to the regional location and distribution of the fat stores. Research has shown that abdominal obesity might have a greater bearing on poor health than BMI alone or fat located subcutaneously.[50, 79, 102, 105]

Epidemiology and Costs

In the last two decades, the prevalence of obesity has increased dramatically in America. Since 1985, the obesity rate in America has doubled among adults, and tripled among children. According to the Center for Disease Control the prevalence rate is currently over 26% in the obese category and 60% of Americans are either overweight or

obese (BMI ≥ 25 kg/m^2).[35] NHANES studies have predicted an even higher prevalence rate at 66.3% overweight and 32.2% obese[163]. In addition, NHANES studies predict that 4.8% of the population is morbidly obese with a BMI over 40.0 kg/m^2.[163] While these statistics are alarming, the rate at which they are increasing is even more so. Just twenty years ago the obesity rate was below 15% with only one in 200 being morbidly obese vs. the current rate of one in three that are obese with one in twenty classified as morbidly obese. The rates have been projected such that by 2030, close to 90% of American adults will be overweight, and 51% will be obese.[211]

With such a rapidly increasing prevalence, obesity undoubtedly has a major impact on American economy and the health of its citizens. It has been estimated that obesity directly cost well over 100 billion dollars per year in America.[87, 218] This number does not take into account the fact that obesity is a risk factor for several other diseases costing billions of dollars a year.[87, 218] Health professionals project that this increase will raise annual health care costs to close to $900 billion dollars.[211]

Comorbidities

It is very well known that obesity has several different repercussions for an individual's health. Obesity has been shown to substantially raise the risk of hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea and respiratory problems, and endometrial, breast, prostate, and colon cancers.[2, 32, 106, 148, 204, 211, 218] An increase of any and all of these conditions affects the mortality of this population. In a large analysis of cohort studies, Allison et al. estimated that roughly 300,000 American deaths are attributable to obesity each year

(1991).[6] It has been shown that a forty-year-old female and male non-smoker will lose 3.3 and 3.1 years of life, respectively, if they are overweight. Those numbers increase to 7.1 and 5.8 years respectively if they are obese.[144] In another study, it was shown that subjects in the highest BMI indexes had a relative risk of death of 2.58 and 2.00 (men and women respectively) compared to those with a 'normal' BMI. In this same study, a high BMI was most predictive of death caused by cardiovascular disease which had a relative risk of 2.90 in men.[32] Willett et al showed that women who have a BMI of 29 kg/m^2 and above had a relative risk of 3.56 for developing coronary heart disease when compared to women with a BMI of under 21 kg/m^2.[216] A recent study showed that an increase of $3kg/m^2$ in BMI raises risk for thromboembolic stroke by 10% to 30%. [162]

Obesity plays such a large role in the development of cardiovascular disease mainly because it raises several of the well-known risk factors for the disease. Obesity has been shown to increase blood pressure, diabetes, and LDL cholesterol while decreasing HDL cholesterol, all of which have been significantly correlated with cardiovascular disease.[163] The effect that obesity has on plasma homocysteine levels is a little more debatable. Very little research has examined this topic, and that which has been done has been rather inconclusive. One group found that plasma homocysteine is significantly associated with waist-to-hip ratio, but not BMI.[107] Another group of researchers conducted two separate studies that examined plasma homocysteine levels in hypertensive and diabetic obese/non-obese subjects. In both studies plasma homocysteine levels were significantly correlated to obesity.[99, 100] In a dietary intervention using a prepared meal plan, subjects with total plasma homocysteine levels of 10.8 μM/L lost an average of 4.8 ±3.0 kg of body mass and their homocysteine was reduced by 1.5 ± 3.3

μmol/L ($p<0.01$).[118] This not only indicates that body mass (and therefore BMI) might be correlated with plasma homocysteine levels, but it also suggests that mass loss might be a viable treatment for high plasma homocysteine levels.

CARDIOVASCULAR DISEASE

Incidence and Mortality

It has been well documented that obesity raises the risk of developing cardiovascular disease. While there are many forms of cardiovascular disease, when lumped together, they are by far America's number one killer. Roughly 2,400 Americans die from cardiovascular disease every day. That is more deaths than diabetes, respiratory disease, cancer, and accidents combined.[131] Cardiovascular disease was the underlying cause of death in roughly 36% of all deaths in 2004. However, cardiovascular disease is mentioned in the cause of death in 57% of all deaths that year.[131] The National Center for Health Statistics predicted that if all forms of cardiovascular disease were eliminated, the life expectancy in America would rise by seven years.[9] While the death rates of cardiovascular disease are actually decreasing[131], the actual prevalence of the disease is not. Roughly 15% of men between the age of 55 and 64 have cardiovascular disease, while that number jumps to 35%, 52%, and 69% for the ages between 65-74, 75-84, and 85-94 respectively.[82] While people are living longer and life expectancy is going up, this suggests that more and more people will live with cardiovascular disease. In 2005, it was estimated that close to 80 million Americans had some form of cardiovascular disease. Close to 40 million of those were over the age of sixty. Seventy-three million people either had hypertension, or were being treated for it. Sixteen million people had coronary

heart disease, eight million of whom had a myocardial infarction, and nine million who had angina. Of the eighty million cardiovascular disease patients, five million people had heart failure. An additional six million had a stroke.[147]

Costs

With so many people affected by cardiovascular disease, the economic burden is very high. In 2005, close to seven million inpatient procedures were performed on cardiovascular disease patients.[47] The estimated direct and indirect cost associated with cardiovascular disease is 448.5 billion dollars for 2008. [167]

Risk Factors

There has been an abundant amount of research done to determine the causes and risk factors for cardiovascular disease. Through this research, a well-established list of risk factors has been created. These risk factors are commonly referred to as the 'traditional risk factors.'[56]

TABLE 1.
Traditional Risk Factors for Cardiovascular Disease

Tobacco Smoking	Obesity
Hypercholesterolemia	Insulin Resistance Syndrome
Elevated Plasma LDL cholesterol	Sedentary Lifestyle
Low HDL cholesterol	Age
Hypertriglyceridemia	Menopause

Hypertension	Male Sex
Diabetes Mellitus	Family History

In addition to the traditional risk factors, there is large list of 'nontraditional' or 'emerging' risk factors that have been receiving a lot of attention recently. [56]

TABLE 2.
Emerging Risk Factors for Cardiovascular Disease

Elevated plasma lipoprotein A[165]	ACE gene polymorphism[141]
Elevated plasma lipoprotein B[175]	Increased oxidation of LDL[179]
Small dense LDL[14]	Mutations affecting platelet activation[41]
Paraoxonase deficiency[111]	Elevated plasma fibrinogen[83]
Infectious agents[71]	**Hyperhomocysteinemia [55]**

Elevated plasma homocysteine levels have been shown in dozens of studies to be a strong, independent risk factor for cardiovascular disease. While the nature and mechanisms of the relationship is still debatable, there is ample evidence to provoke further study into plasma homocysteine and its interaction with other cardiovascular disease risk factors.

HOMOCYSTEINE

Biochemistry:

Homocysteine is a sulfur-containing amino acid that is formed during the demethylation of dietary methionine. [99, 100, 138] There are several forms of homocysteine. Free reduced homocysteine accounts for about 2% of the total plasma homocysteine (tHcy) levels in healthy individuals. In homocystinurics (extremely high total plasma homocysteine) free reduced homocysteine may be as high as 20% of the total. Homocysteine can oxidize with itself to create homocysteine. This accounts for roughly 5-10% of tHcy. Homocysteine can also oxidize with cysteine to form homocysteine-cysteine mixed disulfide (also 5-10% of tHcy). The most common (80-90%) form of plasma homocysteine is protein bound homocysteine. Albumin is thought to be the most common protein carrier.[92] Unless otherwise mentioned, a reference to plasma homocysteine is typically referring to total plasma homocysteine.

There are very scarce levels of homocysteine in food sources, so almost all of it is derived as an intermediate in the methionine cycle. [92] Methionine is an essential amino acid that is found in the highest quantities in animal products such as eggs, meat, fish, and milk at levels around 3 g/10 0g of protein. Methionine is also found in most plant sources such as fruit, vegetables, nuts, and cereals at levels around 1 g/100 g of protein. The exceptions to plant sources are peaches and grapes which have a content of 3.6 g/100 g, and Brazil nuts which have the highest content at 5.6 g/100 g. Consequently, a meal very high in protein and methionine (over 50 grams) can elevate plasma homocysteine levels. However, it has been shown that meals containing moderate levels of protein (15-18 grams) do not have an effect on plasma homocysteine levels.[76] It is estimated in healthy people that around 1.32 mmol of homocysteine is exported from cells to the blood each day, but only 0.006 mmol is secreted in the urine. This suggests that the body

is able to metabolize a moderate amount of homocysteine through the methionine cycle and only in rare circumstances when some part of the metabolism process is not working correctly will homocysteine be excreted in urine.[92]

Methionine Cycle

The methionine cycle is shown in Figure 1. Dietary methionine is first introduced in the cycle by the enzyme methionine adenosyltansferase (MAT). MAT catalyzes the conversion of methionine into energy rich S-adenosylmethionine (SAM). Methyltransferases then use SAM as the substrate for methyl-group transfers to a number of different acceptors. Some processes that use methyl groups are synthesis of phospholipids, proteins, DNA, RNA, creatine, and epinephrine creation, as well as gene expression.[92] After SAM donates its methyl group to its acceptor, S-Adenosylhomocysteine (SAH) is formed. SAH is then hydrolyzed to create homocysteine and adenosine by the enzyme SAH hydrolase. This is a reversible reaction; however, it is undesirable as it might lead to excess SAH accumulation. SAH is a strong end-product inhibitor and will impair methyltransferase activity.[92]

At this point, homocysteine serves as a branch point in the methionine cycle. It will either undergo remethylation or transsulfuration.[92] It has been shown that on a normal diet, roughly 50% of the homocysteine goes through remethylation, and the other 50% goes through transsulfuration. [213]

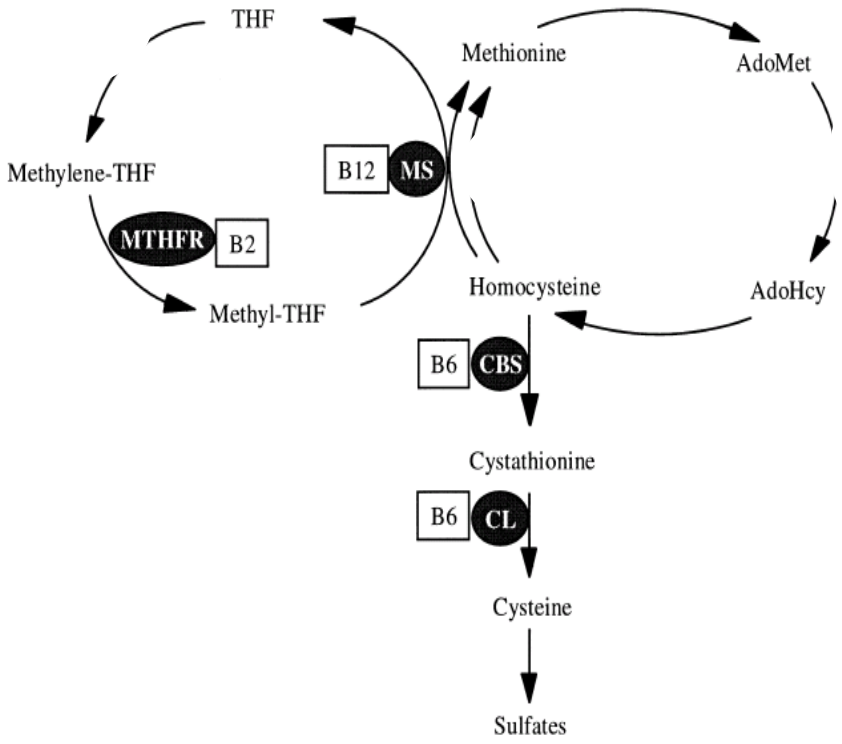
Remethylation

The methionine cycle is completed once homocysteine is remethylated into methionine. The remethylation of homocysteine is done by a second, separate cycle called the folate cycle. Methionine synthase (MS) uses 5-methyltetrahydrofolate as the methyl donor group and methylcobalamin (a coenzyme of vitamin B12) to convert homocysteine back into methionine.[17, 92] It appears that all tissue types have the ability to remethylate homocysteine using this pathway.[17, 58-62, 92]

Transsfulfuration

The transsulfuration pathway uses the enzyme cystathionine synthase (CBS) and vitamin B6 as a co-factor to irreversibly convert homocysteine into cystathionine. Cystathionine is converted into the amino acid cysteine. Cysteine is used in a variety of metabolic pathways or converted into inorganic sulfate and excreted in urine. [92, 213] It appears that the transsulfuration pathway might be limited to certain cells however. For example, it has been shown that there is very little CBS activity in cardiovascular endothelial cells in both rats and humans.[59, 92, 199, 210] Finkelstein and colleagues found that CBS activity is limited to the liver, kidney, pancreas, adipose, brain, and possibly intestinal mucosa. [59] The capacity of the transsulfuration pathway appears to be limited. If the remethylation pathway is impaired for any reason, the transsulfuration pathway is not able to handle the increased plasma homocysteine. [92] Any type of error in the transsulfuration pathway will typically lead to high levels of homocysteine as well as methionine. An error in the remethylation pathway will typically only raise homocysteine levels while methionine levels remain normal or even low. [164]

FIGURE 1.
Homocysteine Metabolism
Derived from Nygard, 1999[138]



Reference Ranges for Plasma Homocysteine

While specific standards have not been unanimously agreed upon, most literature describes a normal plasma homocysteine level somewhere in between 5 and 15 μM/L. [8, 75, 142, 152, 191, 192, 197] However; most studies that exhibit this reference range are positively skewed. [192] According to an analysis done on the Physicians Health Study, the risk for myocardial infarction increased 3.4 fold when the plasma homocysteine level was in the tailed part of the distribution of the curve.[176] This suggests that it may be inappropriate to include the skewed part of the curve in a 'normal' reference range for healthy people. [192]

Reference ranges that are generated using B-12 and folic acid vitamin supplementation, which is a major determinant of plasma homocysteine levels (to be discussed later), lose most of their skewness, suggesting that most cases of high homocysteine can be attributed to a shortage of vitamin B-12 and folic acid. The studies that use this supplementation show that a better reference range for tHcy levels is closer to 5-12 μM/L. [75, 108, 152, 191] Other studies have suggested upper levels as low as 11.7[142], 11.4[166], and 10.5 μM/L.[113]

Plasma homocysteine levels above the normal reference limit, whether that is 12 or 15 μM/L, but below 30 μM/L are given the definition moderate hyperhomocysteinemia (also known as homocysteinemia). Intermediate hyperhomocysteinemia is a plasma level between 30–100 μM/L. Acute hyperhomocysteinemia is any plasma level >100 μM/L.[8] When homocysteine reaches levels this high, it is common that a large amount of homocysteine will be excreted in the urine. This condition is called homocystinuria. Homocystinurics have been known to have plasma homocysteine levels as high as 500 μM/L. [8]

There are many determinants of plasma homocysteine levels, and it has been proposed that separate reference ranges might need to be created for different populations. [192] For example, if a prepubescent child had a plasma homocysteine level of 12 μM/L, that would be considered high because the average for that age is 5 μM/L.[198]

Determinants of Plasma Homocysteine

There are considered five different categories of homocysteine determinants. Genetic, physiological, diseases, drug, and life-style factors all influence total plasma homocysteine levels. [198]

Genetic Factors

Of all the determinants, genetic mutations have the largest capacity to affect plasma homocysteine levels. There are many steps in methionine and homocysteine metabolism, and a defect at any point can lead to hyperhomocysteinemia or homocystinuria.

Cystathionine β Synthase (CBS) Deficiency

In the early 1960s, while searching for metabolic abnormalities as causes of mental retardation, researchers observed that some patients were secreting a large amount of homocysteine in their urine. These researchers found that the defect was the absence or diminished activity of cystathionine synthase.[213] As described above, CBS is responsible for the irreversible degradation of homocysteine. Without CBS, the entire transsulfuration pathway of the methionine cycle is shut off. Since the late 1960s, it has been well researched and shown that homozygous deficiency of CBS is associated with homocystinuria and precocious vascular disease.[74] The effect heterozygosity for CBS deficiency has on plasma homocysteine is not as clear. Fasting plasma homocysteine levels in these individuals is typical normal or slightly elevated. After a methionine load, however, plasma homocysteine will raise beyond normal levels. [27, 198]

Elevated plasma homocysteine levels due to CBS deficiency can be treated in roughly 50% of patients with pyridoxine.[129] In patients non-responsive to pyridoxine, oral betaine is normally effective at lowering plasma homocysteine levels.[215]

MTHFR Deficiency

As described above, 5-methyltetrahydrofolate is the methyl donor to convert homocysteine back into methionine. The enzyme methylenetetrahydrofolate reductase (MTHFR) is required for this demethylation to occur. Severe deficiency of MTHFR will result in homocystinuria, while milder deficiencies of the enzyme are only associated with mild to moderate hyperhomocysteinemia.[164] These deficiencies are most often the result of a C677T polymorphism of the enzyme. Homozygosity for this condition will impair folic acid status and provoke hyperhomocysteinemia.[198] However, there have been eighteen other rare MTHFR mutations identified. The C677T polymorphism is the only one that has consistently shown to influence plasma homocysteine levels. In North America, it has been shown that 11%-15% of the population is homozygous for C677T mutation.[67, 164] As mentioned previously, this specific mutation will interfere with folic acid metabolism and can be expected to lower plasma folic acid levels. This decrease in folic acid will in turn raise plasma homocysteine levels to a mild or moderate hyperhomocysteinemia level. Normally, the folic acid levels in these individuals are not considered in the deficient range, but rather in the low end of the 'normal range.'[164] There have been several studies examining the relationship between the C677T mutation and cardiovascular disease. These studies have shown that there is a significant correlation

between the mutation and coronary artery disease [128], cerebral infarction [127], and venous thrombosis.[13, 116]

In contrast, there have been studies that have shown no correlation between C677T mutation and cardiovascular disease.[96, 97, 109, 117] These discrepancies might be explained due to several of the studies not assessing folic acid status or having a large enough of a sample. [164]

Despite the discrepancies, a meta-analysis concluded that the mutation of MTHFR was a modest yet still significant risk factor for cardiovascular disease.[97] Treatment for this mutation has proven to be relatively easy. Since folic acid status is what decreased levels of MTHFR, it has been found that folic acid supplementation in these patients is an effective way to overcome the effect of the mutation and restoring normal plasma homocysteine levels. [164]

MS Deficiency

As described previously, methionine synthase (MS) is the other enzyme crucial to the remethylation of homocysteine into methionine.[17, 92] Methionine synthase is found in practically all mammalian tissues. MS is the enzyme responsible for demethylating methyltatrahydrofolic acid. MS is dependent on a cofactor, methylcobalamin, which is derived from vitamin B-12. Severe mutations of MS are correlated with severe hyperhomocysteinemia and homocystinuria. There have not been any polymorphisms found that lead to only moderate hyperhomocysteinemia. Deficiencies of methionine synthase result most commonly from defects in the enzyme itself, or indirectly by impaired cobalamin transport proteins. [17, 134, 198]

Physiological Determinants

Plasma homocysteine levels increase throughout life in both men and women. As mentioned previously, the average total plasma homocysteine level in prepubescent children is 5 μM/L for both boys and girls.[198] During puberty, plasma homocysteine levels will typically increase to 6-7 μM/L. This change is more pronounced in boys than it is in girls.[187, 198] The characteristically skewed distribution also takes form during this stage in life.[187] After puberty, plasma homocysteine increases 3-5 μM/L.[198] Data from NHANES III shows that plasma homocysteine is 40% (3.6 μM/L), 18% (1.6 μM/L), and 2.2% (0.2 μM/L) higher in adults over seventy years old, between fifty and seventy years old, and between thirty and fifty years old, than it was in subjects younger than thirty, respectively. [68] The mechanisms that lead to this age-related increase in plasma homocysteine are not well understood, but it is believed that impaired renal function is involved. [155, 56, 79] It has also been proposed that this increase with age might be due to diminished cystathionine β Synthase activity. [70]

In adulthood, plasma homocysteine levels are typically 1-2 μM/L higher in males than in females (pre-menopausal). [198] Using data from NHANES III and the Hordaland cohorts, it was shown that plasma homocysteine was 21.1% higher in men than in women (1.9 μM/L). [68, 198] This disparity might be explained by differences in muscle mass, hormones, and vitamin status. [155, 156] Plasma homocysteine levels in post-menopausal women tend to be higher than pre-menopause and typically will resemble that of men the same age.[134, 198]

Pregnancy has been shown to substantially lower plasma homocysteine (about a 50% reduction). During the first and second trimesters, plasma homocysteine will decrease, and then stay relatively stable for the remainder of the pregnancy. Normally, typical plasma homocysteine levels will be attained two to four days post-partum. [11] Maternal plasma homocysteine is inversely related to neonatal mass and gestational age after delivery, which suggests that the decrease during pregnancy might be due to fetal uptake of maternal plasma homocysteine.[114, 198] Another hypothesis is that lower plasma homocysteine levels are a physiological adaptation which supports placental circulation. [23, 198]

Drug and Disease Determinants

There are a number of different drugs and diseases that can affect plasma homocysteine levels. It appears that most of these drugs and conditions that effect plasma homocysteine levels have a common variable: they interfere with the individual's vitamin status. [198] Vitamin status will be discussed more thoroughly in the lifestyle determinants section, but it is well known that low levels of the vitamins associated with homocysteine metabolism will affect plasma homocysteine levels accordingly.

Drugs

Methotrexate is used for cancer chemotherapy, leukemia, psoriasis, as well rheumatoid arthritis patients. It is considered an antifolate drug, and in turn, can increase plasma homocysteine levels up to 100 μM/L. [126, 151, 154, 157] Several other drugs also

interfere with folic acid status, such as phenytoin (anticonvulsant) and colestipol (bile sequestrant). [198]

Drugs that interfere with vitamin B-12 have also shown to increase plasma homocysteine levels. Cholestyramine [45], histamine H2-receptor antagonists [66], omeprazole [20], as well as metformin, have all been shown to interfere with B-12 absorption. However, increased plasma homocysteine levels have only been reported in cholestyramine and metformin. These two drugs might also affect folic acid absorption. [34, 198] Many other drugs have the potential to alter plasma homocysteine levels; for a complete list, see Ueland, 2000. [198]

Diseases

Similar to drugs, any disease that negatively affects the absorption or function of the vitamins crucial to homocysteine metabolism will cause plasma homocysteine to rise. Some of these conditions are: renal failure [49], leukemia [151], and psoriasis. [154] Other conditions that have been reported to affect plasma homocysteine levels are diabetes (type I) [89], hypo/hyperthyroidism [132], and heart transplant patients. [22]

Life-Style Determinants

While genetic disorders have the greatest potential to cause severe hyperhomocysteinemia, those conditions are generally rare and account for only a fraction of cases of elevated plasma homocysteine levels. With the exception of vitamin B-12 deficiency, life style determinants do not have the same ability to drastically increase plasma homocysteine levels. Instead, they are the most common cause of mild

to moderate hyperhomocysteinemia.[198] As discussed previously, the general populations' homocysteine distribution is positively skewed. It has been shown that the majority of the individuals in the 'tail' of the distribution have some sort of vitamin deficiency.[193] Indeed, studies including vitamin supplementation exhibit a more symmetrical distribution. [152, 191] This evidence suggests that most cases of elevated plasma homocysteine levels are directly related to poor nutrient status.

Protein and Methionine Consumption:

As discussed earlier, there is very little homocysteine in dietary sources. Practically all homocysteine is derived directly through the methionine cycle. With that in mind, it would seem obvious that a diet high in methionine would, in turn, increase plasma homocysteine levels. Indeed, plasma homocysteine levels have been shown to increase 14% eight hours after a protein (source of methionine) rich meal. [76] With that said some studies show that homocysteine levels do not seem to be related to daily dietary methionine or protein content. [10, 171] It appears that the methionine cycle is able to adequately handle the typical diet, and although a large protein-rich meal will cause a spike in plasma homocysteine, these changes are temporary, and levels return to normal after an overnight fast. [203] There have been other reports that a high protein diet can actually decrease fasting plasma homocysteine. It is hypothesized that a high daily protein intake leads to more efficient catabolism of homocysteine through greater activation of enzymes in the methionine cycle. In addition, high protein foods contain other amino acids and vitamins that could influence homocysteine metabolism. [182] In

animal studies, it has been shown that mainly the transsulfuration pathway is activated in the animals on a high methionine diet. [59]

Vitamin B-6 Status

Vitamin B-6 is used as a co-factor in the transsulfuration pathway and a deficiency in this nutrient has the potential to increase levels of plasma homocysteine. The metabolite effecter SAM is one of the ways the body controls the methionine cycle. When methionine levels are high, SAM activates the transsulfuration pathway, and inhibits the remethylation pathway. [92, 123] This limiting mechanism will dictate how much vitamin B-6 is able to effect plasma homocysteine levels. Since B-6 is used only in the transsulfuration pathway, as long as methionine, and thus SAM, levels are low, such as in a fasting state, plasma homocysteine will not be affected. Since most of the time tHcy is measured while fasting, vitamin B-6 deficiency does not seem to have an effect on plasma homocysteine levels. However, if a vitamin B-6 deficient individual takes a methionine load and then has tHcy measured, it will be dramatically elevated. [52, 123]

Vitamin B-12 Status

Cobalamin, also known as vitamin B-12, is the main catalyst in the 5-methtetrahydrofolate conversion of homocysteine into methionine. Without cobalamin, the entire remethylation cycle essentially gets shut off.[92] Vitamin B-12 deficiency is a common cause of moderate to severe fasting hyperhomocysteinemia. [191, 193, 198] It is estimated that a person deficient in cobalamin will have a fasting plasma homocysteine level between 25 and 105, depending greatly on the severity of the deficiency, as well as

the presence of any of the other determinants discussed in this section. [198] Interestingly, women typically have a higher cobalamin status than men. In a study that examined NHANES III participants, women had an average of 463.6 pmol/L, while the men only had 373.3 pmol/L.[68] This difference might play a role in explaining why men have higher plasma homocysteine levels than women. In this same study, they found that plasma homocysteine and B-12 have a strong, negative association.[68]

Folic acid Status

Similar to cobalamin, folic acid is an essential nutrient in the remethylation pathway of the methionine cycle. As discussed previously, tetrahydrofolate is the active substrate in converting homocysteine back into methionine. Tetrahydrofolate is derived from folic acid. Deficiencies in folic acid can lead to moderate to severe hyperhomocysteinemia.[92] While folic acid deficiency has the potential to lead to much higher values of plasma homocysteine, under normal circumstances, it will only raise plasma homocysteine levels to anywhere between 20 and 50 μM/L.[198] In the NHANESIII study, a negative linear association was found between folic acid status and plasma homocysteine levels ($P < 0.0001$ for linear trend), demonstrating that folic acid plays a significant role in plasma homocysteine levels. The multivariate-adjusted average plasma homocysteine level was 34.8% (3.2 μM/L) higher in the participants with the lowest plasma folic acid than those with the highest. Similar to B-12 status, it was found that women have higher folic acid levels than men (by 2.3 nmol/L).[68] Again, this might explain some of the variance of homocysteine levels between men and women.

Smoking

Cigarette smoke, as well as plasma cotinine, has been shown to moderately raise plasma homocysteine. Cotinine is a metabolite of nicotine and is directly related to absorbed nicotine.[21] Cotinine might be a better measure of smoking status because study participants tend to underreport cigarette habits and smokers inhale cigarette smoke differently.[68] In NHANESIII, it was found that cotinine levels were positively correlated to plasma homocysteine levels. People in the highest quartile of cotinine had significantly higher plasma homocysteine than people in the lowest.[68] In the massive (n=18,000) Hordaland Homocysteine Study, one of the most important findings was that there is a dose-dependent relationship between plasma homocysteine levels and the number of cigarettes smoked per day. This relationship was found in all ages and gender groups and was still strong after adjusting for several potential confounders.[155] This dose-dependent association has been demonstrated in several other large cohorts. [46, 73, 93]

A suggested mechanism behind the plasma homocysteine/cigarette-cotinine relationship is that the free radicals in smoke provoke oxidative stress which affects the redox status of homocysteine. [194]

The reversibility of increased plasma homocysteine with cigarette smoke is uncertain. There are some studies that found cessation of smoking decreases plasma homocysteine [178], while others have not.[188] This disagreement might suggest that the relationship between plasma homocysteine and smoking is not (or is only partially) dependent on the actual smoke itself, but rather, is due to other behavioral traits in smokers.[155] Smokers tend to have a diet containing less vegetables, more fat [150], and less vitamins [146] when compared to non-smokers.

Coffee Consumption

Of all the life-style determinants measured in the Hordaland Homocysteine Study, heavy coffee consumption is among one of the strongest. [136] It appears that this relationship is only present in heavy coffee consumers. In the ARIC study, no relationship was found between plasma homocysteine levels and moderate coffee consumption. Only after four or more cups a day was plasma homocysteine elevation present. [182] The Hordaland study found that more than six cups a day will provoke a plasma homocysteine level 2-3μM/L higher than coffee abstainers. Decaffeinated coffee did not raise plasma homocysteine levels, which suggests that caffeine might be the culprit in this relationship. [136] Caffeine's effect on plasma homocysteine might be related to its influence on the cardiovascular system or kidney function.[88, 198] With that said, it is known that heavy coffee consumption is typically related to an unhealthy life-style and poor nutritional status. [91] When examining three different life-style determinants together, it was found that smokers who consumed a high amount of coffee and a low amount of folic acid had anywhere between 3.2-4.8μM/L higher plasma homocysteine than individuals with a contrasting lifestyle. [137]

Alcohol Consumption

Alcohol seems to have a similar effect as coffee on plasma homocysteine levels. In the NHANES III study, mild drinkers (one to thirty drinks per month) had plasma homocysteine levels that resembled that of alcohol abstainers. At higher levels, alcohol consumption was positively correlated with plasma homocysteine levels. There was also

a correlation between the type of alcohol consumed and plasma homocysteine levels. Both beer and wine were not correlated to plasma homocysteine, while hard alcohol was.[68] In another study, alcohol consumption forms a U-shaped curve with plasma homocysteine reduction up to fourteen drinks per week. Above that level, plasma homocysteine levels began to rise. [155] In a recent study, it was shown that alcohol consumption could have detrimental effects on both folic acid and B-12 status. [72] This suggests that alcohol consumption might raise plasma homocysteine levels by hindering the remethylation pathway in homocysteine metabolism.

Exercise

While the relationship is obscure and still debatable, there have been several studies showing a weak relationship between exercise and plasma homocysteine levels. This relationship was the most pronounced in the elderly, showing a 1 μM/L difference between sedentary and exercising subjects. Exercise was shown to reduce some of the skewness in the distribution curve, which might suggest that it affects mainly those with hyperhomocysteinemia. [139] The inverse relationship between physical activity and plasma homocysteine is not seen in all studies. [46 90] In the Hordaland study, an influence of BMI was observed as the subjects with a low BMI had an inverse relationship between plasma homocysteine and exercise, while those with a very high BMI had a positive relationship. [139] It has not been shown if exercise directly influences plasma homocysteine or if the association reflects healthy lifestyle differences. [155] The relationship between plasma homocysteine and exercise is unclear and more research needs to be conducted to confirm any results.

Obesity

As stated previously, the relationship between obesity and plasma homocysteine is currently obscure. There have not been many studies examining this topic; however, those studies that have been completed have come to different conclusions. Although obesity is a well-known risk factor for cardiovascular disease, it is uncertain if obesity also affects the homocysteine metabolic pathway. Currently, the findings are equivocal for this.

In a study comparing obesity, hypertension, and plasma homocysteine levels, it was found that obesity had a positive correlation with plasma homocysteine. The average plasma homocysteine level in the non-obese normotensives was 8.81 μM/L. The obese normotensives had an average level of 10.71 μM/L, which was significantly higher ($p < .001$) than the non-obese. Within the hypertensives, the same trend was observed. The non-obese had an average of 8.85μM/L while the obese had an average of 14.85μM/L, which was also significant ($p < .001$).[99]

The same research group did a similar study in diabetic patients with similar results. The healthy control group had an average plasma homocysteine level of 8.5μM/L. The non-obese diabetic patients had an average of 10.4μM/L, while the obese diabetics had an average of 13.2μM/L, both of which were significantly higher than the control group ($p < .001$). This suggests that obesity, as well as diabetes, might be causes of elevated plasma homocysteine levels.[100]

In a study of cardiovascular disease patients, it was found that both BMI and waist-to-hip ratio (WHR) were significantly correlated with plasma homocysteine

levels.[37] Another study of cardiovascular disease (coronary heart disease in this instance) patients confirmed that WHR was significantly correlated to plasma homocysteine, but failed to do so with BMI. The researchers suggested that a possible explanation would be the detrimental effects that central obesity has on the cardiovascular system. [107]

In a German study of 500 healthy participants, it was found that plasma homocysteine concentrations of the total study group correlated positively with lean body mass (LBM) ($p < .0001$) but not fat mass, or BMI. [153] These results were confirmed findings from another large European Cohort that found a positive correlation between LBM and plasma homocysteine levels, but not with BMI. [51] In a third study that had very similar findings, the authors suggested that LBM might be the main variable that affects the male-female differences in plasma homocysteine levels.[18] It is suggested that the relationship between LBM and homocysteine is attributable to an increased protein mass relative to body size which could increase circulating methionine and homocysteine. Creatine synthesis is one of the most important reactions that require methyl groups from methionine. The greater amount of methionine that donates its methyl groups, the more homocysteine will be produced.[18]

It appears that most of the studies done on healthy volunteers show no correlation between BMI, fat-mass, or obesity and plasma homocysteine, while studies done on diseased populations do display this connection. It might be the case that some of the conditions mentioned are confounders in the plasma homocysteine/obesity relationship. However, it is clear that the relationship between obesity and plasma homocysteine needs to be investigated further.

HOMOCYSTEINE AND CARDIOVASCULAR DISEASE

Though there is no known biological role of homocysteine, elevated levels of this amino acid have been shown to be an emerging risk factor for cardiovascular disease.[56, 77] This topic is debatable in the literature today, as some incredibly compelling studies have shown that plasma homocysteine is an independent risk factor for cardiovascular disease. On the other hand, further studies have found that the relationship is more casual and that plasma homocysteine is merely a marker of cardiovascular disease.

Homocystinuria and Cardiovascular Disease

As discussed previously, homocystinuria is a severe elevation of plasma homocysteine levels. In the late 1960s, reports of homocystinuria in children with thromboembolisms and arteriosclerosis led researchers to believe that plasma homocysteine is an instigator of vascular and thrombotic disease.[119] Though it is still debatable if moderately elevated plasma homocysteine levels are an independent risk factor for cardiovascular disease, it is very well agreed upon that homocystinuria levels are a risk factor. Even the researchers that seem to side with the argument that mild hyperhomocysteinemia is just a marker of cardiovascular disease, agree that homocystinuria plays a direct role in vascular and thrombotic disease.[124] One researcher went as far as to say, "This condition is unquestionably associated with precocious atherosclerosis and extensive arterial thrombosis."[40] The most common causes of death and morbidity in patients with homocystinuria is thromboembolisms, followed by strokes, peripheral arterial thrombosis, and heart attacks.[40] The findings of patients with

homocystinuria are different than patients with hyperlipidemia. Homocystinuria patients will have a loosening of the internal elastic lamina, intimal hyperplasia, and narrowing of the arterial lumen.[120, 138, 196] Furthermore, patients with homocystinuria have been shown to develop drastic arterial intimal thickening and fibrous plaques.[104, 119, 158] The proliferation of these fibrous plaques is caused by hyperplasia of smooth muscle cells and deposition of collagen fibers. Deposition of cholesterol and lipids in these patients is only occasionally observed.[121] It has been shown that if left untreated, 50% of homocystinuria patients will experience a thromboembolic event and 20% will die before the age of thirty.[130, 138] On the other hand, in a study it was shown that if homocystinuria treatment can reduce plasma levels below 20 μM/L, vascular events can be drastically reduced. Throughout the course of the study[214], only two vascular events occurred after lowering plasma homocysteine levels. Surprisingly, it was estimated that without the plasma homocysteine lowering treatment, twenty-one events would have occurred.[214] Rapid onset of severe cardiovascular disease has been shown after injecting large quantities of homocysteine into laboratory animals.[81] This experiment suggests that homocysteine itself might have a damaging effect on blood vessels.[186]

Hyperhomocysteinemia and Cardiovascular Disease

Since the original literature on this topic came out in the 1960s, a great deal of attention has been focused on determining the relationship between plasma homocysteine and cardiovascular disease. Hundreds of studies have followed, most of which have been done in the last fifteen years.[138] While these studies have shown an undeniable relationship between the two factors, the exact nature of that relationship is still uncertain.

The common belief, after the 1960s studies, was that plasma homocysteine was an independent risk factor for cardiovascular disease. This belief was coined the "homocysteine hypothesis of arterioscerosis."[40]

A majority of early studies strongly support the homocysteine hypothesis. These studies found that plasma homocysteine was a strong and independent risk factor for cardiovascular disease.[26, 80, 158, 195] Boushey et al conducted a classic meta-analysis that included three prospective and six population-based case-control studies that were considered high quality. An additional five cross-sectional and thirteen other case-control studies were also incorporated.[26] This analysis found that for a 5 μM/L increment in plasma homocysteine, the odds ratio for CAD was 1.6 in men and 1.8 in women. This risk elevation is similar to what is seen with a 20 mg/dL increase in total cholesterol. The authors predicted that roughly 10% of the populations risk for CAD can be attributed to high plasma homocysteine. In addition, the odds ratio for cerebrovascular disease at a 5 μM/L plasma homocysteine elevation level was 1.5.[26] This study investigated the effect of increased folic acid consumption and found that approximately 200 micrograms/day reduced plasma homocysteine levels by 4 μM/L, while it also predicted that 13,500-50,000 CAD deaths could be avoided annually with widespread folic acid supplementation.[26] A more recent study found that a high dosage of folic acid (5-10 mg/day) will reverse endothelial dysfunction in CAD patients.[125] The Boushey et al analysis found that plasma homocysteine should be considered an independent graded risk factor for cardiovascular disease.[26]

A more recent meta-analysis found that people with lower than normal plasma homocysteine levels have a 11% lower heart disease risk and a 19% lower stroke risk

after adjusted for all potential confounding variables.[3] A separate finding estimated that a reduction of plasma homocysteine by 3 μM/L would reduce the risk of cardiovascular disease by as much as 16%.[207]

The other school of thought that opposes the homocysteine hypothesis believes that plasma homocysteine is a mere marker of cardiovascular disease. The main reasoning behind this belief is that retrospective studies tend to show stronger associations between plasma homocysteine and cardiovascular disease than prospective studies. While there have been several prospective studies[12, 25, 145, 161, 208] that have shown a strong correlation between the two factors, most large, well conducted prospective studies show weaker[38, 73, 176, 177, 196] or no association at all between plasma homocysteine and cardiovascular disease.[57, 65] The idea that retrospective studies have more consistently shown positive associations between plasma homocysteine and cardiovascular disease, while prospective studies have been less consistent might point to the conclusion that plasma homocysteine is merely an indicator, as opposed to an instigator of cardiovascular disease.[40, 101, 124]

While there is still some debate about what comes first, high plasma homocysteine or cardiovascular disease, it is widely accepted that once a patient has cardiovascular disease, plasma homocysteine plays a very large role in mortality and reoccurrences of vascular complications. A study conducted by Nygard et al followed 600 CAD patients over the course of five years. The patients that had the highest baseline HCY level had less than a 70% survival rate while over 90% of those with the lowest baseline plasma homocysteine levels were still alive. It was determined in these patients

with cardiovascular disease that plasma homocysteine was the strongest modifiable determinant of mortality.[138]

Mechanisms

While there seems to be strong evidence supporting and opposing the "homocysteine hypothesis of arteriosclerosis," it is well agreed upon that there is a relationship between plasma homocysteine and cardiovascular disease. In order to investigate this relationship, it is important to examine the biological plausibility, as well as the mechanisms in which plasma homocysteine could possibly relate to cardiovascular disease.

Cytotoxicity

Extremely high levels of plasma homocysteine have been associated with direct toxicity of endothelial cells in several *in vivo* and *in vitro* studies.[138] These studies are supported by a finding of an increase in circulating endothelial cells in cardiovascular disease that were given a methionine load.[86]

Inflammatory Response

It is well established that atherosclerosis is at least in part due to chronic inflammation.[104, 138] There have been many published findings that show that plasma homocysteine enhances the production of several inflammatory cytokines.[104] Human monocytes express a number of different pro-inflammatory cytokines.[183] In the Physicians' Health Study, plasma homocysteine was significantly correlated to the concentration of intercellular adhesion molecule ICAM-1.[160] In other findings, when

human monocytes were treated for three hours with various concentrations of plasma homocysteine, it was shown that Tumor necrosis factor-alpha, Interleukin 1 beta, Interleukin-6, Interleukin-8, and Interleukin-12 expressions were significantly enhanced 1.2-2.0 fold.[183] Monocyte chemoattractant protein 1 (MCP-1) is known to enhance binding and recruitment of monocytes to the sub-endothelial cell space. MCP-1 has also been shown to increase in cultured human endothelial and smooth muscle cells with an increased homocysteine level.[209]

Oxidative Stress and Endothelial Function

Homocysteine contains a thiol group that is readily oxidized to create several types of reactive oxygen species (ROS).[15, 104] It has been proposed that the ROS will react with endothelial nitric oxide (NO) (a potent vasodilator) and decrease its' availability. With a smaller amount of NO available, the endothelium dependent vasodilatation will be impaired.[15, 104, 138] Endothelium function is considered to be a sensitive marker of vascular pathology and has been shown to be impaired in cardiovascular disease patients.[125] Endothelium function is easily measured using a technique called Flow Mediated Dilation (FMD). This technique provokes intentional ischemia using a blood pressure cuff of some sort on the arm to provoke a hyperemic condition downstream. The pressure is then released and ultrasound technology is used to measure vessel diameter before and after the increased flow.[125] When FMD was measured in children with homocystinuria, it was found to be significantly impaired (2.8% response) when compared with controls (9.0% response).[36] Another recent study confirmed that CBS deficient patients with severe hyperhomocysteinemia had impaired NO-mediated FMD,

compared to matched controls.[125] This relationship is seen even in more moderate forms of hyperhomocysteinemia. Fourteen age and sex matched patients with an average of 34.8 μM/L plasma homocysteine had impaired (6.5% response) vasodilatation compared to controls (10.8% response).[219]

Smooth Muscle and Collagen Proliferation

As described previously, hyperhomocysteinemia and homocystinuria patients have been shown to exhibit fibrous plaques in atherosclerosis, opposed to the traditional cholesterol and lipids seen in the lesions of cardiovascular disease patients with normal plasma homocysteine levels. A good deal of this plaque is created by the growth of smooth muscle cells and collagen in the vessel wall.[104, 138] It was shown in an *in-vitro* study that when exposed to homocysteine, the proliferation of smooth muscle cells was double that of in non-exposed cells.[185]

TREATMENTS FOR OBESITY

As the obesity epidemic is rapidly growing, it is imperative to examine methods to combat and treat obesity. There are numerous different mass loss programs in America today. Although they vary, programs or techniques can be lumped into one of six different categories.

1) Diet therapy aims to provoke mass loss by restricting calories consumed on a daily basis. Low calorie diets (LCD) will typically aim at reducing calories to 1000-1500 per day.[1] Very low calorie diets (VLCD) restrict calories even further to 400-800 per day. [205]

2) Exercise therapy aims to reduce mass by increasing the amount of energy expended. [1, 2]

3) Behavioral therapies include strategies that aim to reinforce diet and exercise therapies. Some of these therapies are cognitive restructuring, self-monitoring, stimulus control, stress management, and social support.[1, 2]

4) Combined therapy is using more than one of these strategies simultaneously. This has been shown to be one of the more effective ways to lose mass.[1, 2] In a review of fifteen RCT's, there was strong evidence that combination therapy produces greater mass loss than diet or physical activity alone.[1]

5) Pharmacotherapy uses FDA approved drugs as an adjunct to any of the above therapies. Typically, doctors will not prescribe pharmacotherapy unless combined therapy has been unsuccessfully implemented for at least six months. A BMI of above 30 kg/m^2 is general criteria to receive pharmacotherapy, but doctors can prescribe it at lower BMI levels with one or more of the following: hypertension, dyslipidemia, cardiovascular disease, type II diabetes, or sleep apnea.[1, 2]

6) Mass Loss surgery is often considered the last resort therapy for severe obesity. Surgery is generally reserved for patients who have unsuccessfully tried the above therapies and have a BMI of over 40 kg/m^2. Patients with a BMI of above 35 kg/m^2 sometimes qualify for surgery if co-morbid conditions are present. Most authorities agree on these standards and will only prescribe surgery if the patient is suffering from the complications of obesity. [1]

Though combined therapies are effective at decreasing mass acutely in overweight and moderately obese subjects, very rarely does it lead to prolonged and maintained mass loss. RCT's suggest that the mass lost will usually be regained unless some sort of maintenance program is continued indefinitely. [1] Several studies have indicated that combined therapy, in the severely obese, has limited long term effectiveness for the vast majority (>90%) of those who attempt to lose mass.[205, 212] The National Institute of Health (NIH) Consensuses Panel claims, "Although a very-low-calorie diet used under close medical supervision is often effective in the short-term treatment of clinically severe obesity, these diets alone generally have not been successful in achieving permanent mass loss."[4]

In the Diabetes Prevention Program, 1079 obese (BMI = 33.9 kg/m^2) subjects were put into a rigorous diet and exercise program. Despite the intensive individualized guidance, after 2.8 years, the patients had only lost 5.6 kilograms. [98]

In the Swedish Obese Subjects (SOS) study, 627 morbidly obese patients (BMI = 40.5 kg/m^2) underwent conventional treatment (from intensive lifestyle advice to diet and exercise) and were followed for ten years. Though there was a modest initial mass loss, at the end of the follow up period, the group had a mass gain of 1.6%.[173] Though the study could be considered a success in that the patients prevented much mass gain, it established that conventional treatments are typically not able to provide maintained mass loss in the morbidly obese.

In a review article examining the effects of very low calorie diets, a mass reduction of 15-25% was typically observed after three to six months. However, after one year, the average was only 9% mass loss, and after four years it was only 5%. [190] These

results were very comparable to another study that had an average mass loss of 3.0 kg over four years in 1637 patients treated with a placebo and lifestyle therapy.[189]

Even pharmacotherapy, which is considered the second line of therapy, has shown to only provide moderate long-term mass reduction in the morbidly obese. In the Swedish Xendos trial mentioned above, the group treated with the actual drug had a mass loss of only 5.8 kg over the 4 years.[189] The NIH Consensuses Panel released the following statement about pharmacotherapy, "Experience with drug therapy for clinically severe obesity has been disappointing. Although pharmacologic studies of anorexigenic drugs suggest a short-term benefit, prolonged and sustained mass loss has not been proven with these agents." [4]

Marielle Bult summarized all of this information well with the statement, "The long-term effects of diet, exercise, and medical therapy on mass are relatively poor."[31]

Mass Reduction Surgery

While it has been shown that most of the "traditional" mass loss methods are relatively ineffective at treating morbid obesity and maintaining the mass loss, mass reduction surgery has shown promising results. While the obesity epidemic worsens, more and more patients have opted for this option of treatment. Between 1993 and 2003, the number of procedures performed annually rose from under 20,000 to well above 100,000.[180]

There are several different types of mass reduction surgery. The most common are: gastric banding, gastric bypass, gastroplasty, and biliopancreatic diverson. [30] All bariatric surgeries are classified as either restrictive, malabsorptive, or both. Restrictive

surgeries limit the amount of food a patient can consume by shrinking the size of the stomach. Malabsorptive surgeries bypass portions of the small intestines, which is where nutrient absorption occurs. Combination surgeries combine both malabsorptive and restrictive properties.[48] Typically, mass reduction will be greater in malabsorptive and combination surgeries than in restrictive alone.[30] A study done in 2004 estimated that over 90% of all procedures performed that year were Roux-en-Y gastric bypasses[133], which is considered mainly restrictive, but partially malabsorptive as well.[48]

Depending on the institution and procedure performed, these surgeries generally cost anywhere from $20,000 to $50,000, which includes the surgery itself plus any future costs that could be attributed to the surgery. Additionally, the cost of the surgery may or may not be covered by insurance. [180]

While the varying procedures have different results in regards to mass reduction, it has been estimated that mass reduction surgeries as a whole will produce a twenty to fifty kilogram loss. As mentioned before, this is compared to a small mass gain seen in medically treated control patients.[30, 112 44]

As described previously, the main criterion for being a surgery candidate is having a BMI over 40 kg/m^2, or 35 kg/m^2 with a high-risk condition. Depending on the surgical center, there are some additional criteria that are often imposed. Some of these criteria are: an absence of medical or psychological contraindications, strong motivation to comply with post-surgery regimens, and a good understanding of the risks involved with the surgery.[48] There are several psychiatric disorders that are relatively common in the morbidly obese and have been shown that, when present, less mass loss than normal can be expected. Some of these conditions are Axis I or Axis II disorder, disturbed eating

habits (binge eating), substance abuse, low socioeconomic status, limited social support, and unrealistic expectations of surgery. [48, 200]

ROUX-EN-Y GASTRIC BYPASS SURGERY

As mentioned in the previous section, the roux-en-Y gastric bypass is by far the most popular of the mass loss surgeries. The surgery is almost always done laparoscopically. Though the surgery was initially performed by opening the abdominal cavity, the minimally invasive laparoscopic procedure has been shown to greatly reduce complications after surgery. [85, 143, 168] In a study examining the outcomes of open RNYGB compared to laparoscopic RNYGB surgeries preformed in 2005 and 2006, it was found that practically every category of negative occurrences was statistically less in the laparoscopic group. The open group had .79% death rate within the first thirty days of surgery while the laparoscopic group had .17%. "Major" occurrences such as deep wound infections, organ space infections, pneumonia, renal failure, and many more were over twice as common at 7.4% in the open group compared to 3.4% in the LRNYGB group.[103]

Surgery Description

The actual procedure involves creating a small gastric pouch (typically around 15-50mL) and separating it from the rest of the stomach to severely restrict the amount of food the patient can eat. The pouch is then anastomosed (joined) with the proximal jejunum. This segment will form the "roux" limb. The other side (distal) of this limb, along with the duodenum, will be anastomosed with the distal jejunum. This will lead to a bypass of a portion of the jejunum, and thus provoking malabsorption in the small

intestines.[31, 85, 115, 168] For a complete description of the surgical procedures, see Higa, 2001.[85]

Mass Loss

Though surgery results, in regards to mass lost, vary from study to study, it is expected that patients who undergo RNYGB will lose around 60-70% of their excess mass in one to two years.[64, 168] Excess mass is the actual mass prior to surgery minus the "ideal mass" for that individual. If the patient does not lose at least 50% of their excess mass, or keep it off for the course of the study, it is deemed a "mass loss failure."[64] Other studies suggest that the surgery typically provokes somewhere between a 30% and 40% mass loss of total body mass in the first year.[168]

It has been shown that it is very common for the patient to gain some of this mass back over the course of time. Mass loss is usually maximal after one to two years after which mass will gradually increase until eight to ten years. Typically after ten years, body mass will stabilize.[31, 168, 174] There can be many causes of mass regain in these patients. The most common causes are an initial large pouch (.30cc), and initial large stoma (.14mm), a dilated pouch, dilated stoma, staple line disruption, and increased energy consumption. On average, roughly 15% of patients will regain enough mass to have "mass loss failure."[64]

Despite the relatively small percentage of patients that regain a large amount of mass, the majority of patients maintain considerable mass loss for at least ten years (very few studies follow patients beyond this time frame). In a recent meta-analysis, it was found that nearly all studies show at least an average of 50-60% excess mass lost at the

end of the follow-up period. One of the studies had a follow up of sixteen years, two had a follow up of fourteen years, one had a follow up of ten to twelve years, and four had a follow up between four and seven years.[168] With such long term follow up periods, it seems clear that for the majority of patients, the RNYGB surgery is a very effective way to significantly reduce body mass and maintain that mass loss for an extended period of time.

Side Effects

Despite the significant mass loss that occurs with this surgery, one has to remember that it is still surgery and has its risks. It is very well known that any type of gastric surgery has many complications associated with it. Some of these complications are common and relatively mild, while others are rare, but very serious. Most of the time, the benefits of the surgery in mass reduction and improved health (to be discussed later) outweigh these risk for the patient.

The following have been reported as rare complications that occurred in less than one percent of 1040 patients treated in one surgical center: staple line failure, stenosis at mesocolon, bleeding requiring transfusion, death, incomplete division of the stomach, pulmonary embolism, trocar hernia, deep venous thrombosis, pneumonia, and wound infections. The following were reported in one through five percent of patients in the same study: stenosis at gastrojejunostomy, internal hernias, gallstones, and marginal ulcers. The total complication rate for this study was just under fifteen percent.[84] A different study examining 4,631 LRNYGB patients found similar results with most complications occurring under one percent of the time. [103] With that said, when looking at

all complications grouped together, that study found that roughly seven percent of patients will experience some sort of complication in the first thirty days. Close to four percent of patients will have to return to the operating room due to one of the complications.[103]

However, another study examining 235 RNYGB patients found a few more complications. Over twenty three percent of the patients in that particular study reported having some sort of complication.[143] There could be a couple of reasons for this discrepancy. The first possibility could be is how the studies defined a complication, as well as how long the patients were followed. The study that had only a seven percent complication rate was only found in the first thirty days. This study also did not seem to report the less severe complications such as nausea, vomiting, dehydration, or the dumping syndrome.[103] On the other hand, the study that had a twenty three percent complication rate had an average follow up of over twelve months and included complications such as prolonged postoperative stay, dehydration, as well as nausea/vomiting. When this study separated their "early complications" from their "late complications," they found just above nine percent occurred in the "early" phase[143], which is much closer to that which was found in the first study.[103]

Some of these "less serious" complications of surgery seem to be rather common. The dumping syndrome, which is a group of symptoms that include facial flushing, lightheadedness, palpitations, fatigue, and diarrhea can occur in up to 70% of patients. This syndrome is usually provoked by consuming foods high in sugar.[181] Up to 50% of patients can experience nausea and vomiting. These symptoms are usually the result of eating too much, too quickly.[122]

Vitamin Deficiencies

The whole concept behind the malabsorptive properties of the RNYGB surgery is to not allow enough time for the nutrients to fully absorb in the digestive tract, and therefore provoke mass loss by providing less energy for the body to use. This is a good thing when it comes to mass loss, but along with the malabsorption of energy containing nutrients, the other nutrients essential for life functions also do not get absorbed at their normal rate. This malabsorption often leads to a deficiency in particular nutrients. More importantly, this situation is aggravated by the restrictive properties of the surgery, which decreases the total amount of nutrients that could potentially be absorbed.[43] The most common nutrient deficiencies in RNYGB surgery are vitamin B-12, iron, and thiamine. [115] A less common, but still possible, deficiency resulting from surgery is folic acid.[115]

Vitamin B-12

Vitamin B-12 (cobalamin) deficiency has been defined as a plasma level under 150pg/ml-200pg/ml.[168, 202] Cobalamin deficiency is rather rare in the general population because it is widely available in animal products. Additionally, it is stored in large quantities in the liver.[159] That being said, it is quite common in RNYGB surgery patients to have this vitamin shortage. While cobalamin deficiency has been seen in as many as 64% of surgery patients [78], it is more commonly observed in 20-30% of patients, depending on the limit definition used, as well as the presence of any type of post surgery supplementation.[168] Some possible factors that lead to B-12 deficiencies include achlorhydria, which prevents B-12 from being separated from food, and poor production

or secretion of intrinsic factor, which is needed for the nutrient to be absorbed.[43, 159, 168] Post-surgery, some patients become intolerant of meat and dairy, which are key sources of B-12 and will contribute to its deficiency as well.[115, 168] Another major factor resulting in B-12 deficiency is that other chemicals that release cobalamin from food, such as hydrochloric acid and pepsin that reside in the stomach, are rarely found in the small gastric pouch created during surgery. This makes the absorption of *protein bound* cobalamin difficult.[159] Absorption of crystalline B-12 does not seem to be affected by the surgery[159], which is why sufficient supplementation will usually restore cobalamin levels.

Supplementation has been shown to be affective at preventing cobalamin deficiencies in RNYGB patients. A study found that roughly 350 μg of crystalline cobalamin was necessary to prevent deficiency post-surgery.[159] Most studies examined recommend 500-600 μg to sufficiently cover this amount. [29, 159, 168] It was found that this recommended amount will raise over 80% of deficient patients into the normal range.[29] If the patient does not respond to oral supplementation, 1000-2000 μg administered intramuscularly once every month will correct the majority of the remaining 20%.[115, 168, 202]

Folic Acid

While not as common as B-12 deficiency, folic acid deficiency is equally important in the metabolism of homocysteine and needs to be addressed if present in post-surgical patients. Folate is a generic term for a water-soluble B-complex vitamin. It is essential for DNA synthesis, erythrocyte formation, and a cofactor in many other

metabolic pathways apart from homocysteine. Maintaining sufficient levels of folic acid is important because deficiency can also lead to megaloblastic anemia.[115]

Folic acid is preferentially absorbed in the proximal portion of the small intestine.[168] For this reason, folic acid status might be strained after surgery because this is the part of the intestine that gets bypassed. However, it has been shown that the small intestines are able to adapt following surgery and are able to absorb folic acid along the entire small intestine.[7]

Folic acid deficiency is normally defined by any level under 3 ng/mL.[202] The normal range for plasma folic acid levels is 6-25ng/mL.[115] Deficiency is seen in 9-35% of surgeries.[28] In a recent study, the mean folic acid level of all patients continually rose for each follow-up visit. After three years the mean folic acid level was 12.2 ng/mL compared to 10.8 ng/mL pre-operation.[202] While it appears that folic acid deficiency is typically not a big concern, it is still important to be monitored post-surgery and treated if present. Brolin et al found that a 400 μg supplement of folic acid is adequate to correct almost any deficiency.[29]

Surgical Experience

Another large factor to examine when looking at post-surgery complications is the surgeon's experience. In a ground-breaking study, Flum and colleagues found that early mortality after surgery was directly linked to surgical inexperience with the procedure. Patients that had inexperienced surgeons were nearly five times more likely to die within thirty days of surgery, compared to patients of experienced surgeons. Quantitatively,

surgeons that had less than fifty surgeries had a death rate around six percent compared to less than half a percent for those with 100 or more surgeries in experience.[63]

Health Benefits

Other than mass loss, gastric bypass surgery has typically been shown to drastically improve the patients overall health.

Mortality

In one of the very few studies that examine long-term mortality rates after gastric bypass surgery, Adams et al analyzed data from 9949 surgical patients that received surgery from 1984-2002 in a Utah surgical practice, as well as 9628 matched controls. The matching was based off of sex, BMI, age, and year. Surprisingly, between the two groups, the researchers found the same mortality rate in the first year after surgery between the two groups (0.53% in surgery group compared to 0.52% in the control group).[5] This can be expected from the death rates discussed previously in the complication section above. This study brings to light the idea that the risk of dying from complications post-surgery is very close to the risk of *not* having the surgery at all.

The Adams et al study proceeded to follow the patients for an average 7.1 years (>70,000 person years). After the follow up period, 213 surgical patients had died compared to 321 from the control group. This is the equivalent of 37.6 and 57.1 respectively per 10,000 person years. When the cause of death was accounted for, 150 surgical patients died from some sort of disease compared to 285 controls (26.5 and 50.7 per 10,000 person years). Cardiovascular disease claimed fifty-five deaths in the surgery

group compared to 104 controls. Only two surgical patients died from diabetes, while nineteen controls died from the same disease. More than twice as many people (73 compared to 31) died from cancer in the control group. The only surprising statistic was that there were twice as many deaths (sixty-three compared to thirty-six) caused by non-disease reasons in the surgery group. This figure is greatly influenced by suicide rate, which was three times greater in the surgical group. The authors suggest that a possible explanation for this is that a majority of obese persons have unrecognizable mental health problems that may come to surface after surgery. They recommend that further studies explore this topic more in-depth and provide more rigid pre and post surgical psychological counseling to patients.[5]

In a similar study, another group of researchers found similar results in regards to overall mortality rates. There was a five-year follow-up period of roughly 1000 surgical patients and 6000 controls. Overall mortality rate for surgical patients was .68% compared with 6.17% for the controls. The most notable risk reductions were in cardiovascular disease (including hypertension), endocrinologic conditions (including diabetes), and respiratory conditions.[39]

In the same study that found that experience of the surgeon relates directly to thirty day mortality, it was found that ten year survival of bariatric patients was relatively high at 91.2%. At fifteen years follow-up, 11.8% of the surgical patients had died, compared to 16.3% of non-operated patients.[63]

It appears relatively clear that roux-en-Y gastric bypass surgery is very effective at prolonging life in the morbidly obese. A reason for this extension could be the reduction in many chronic disease risk factors. As discussed previously, it is well proven

that obesity drastically increases many of these risk factors, so it would seem probable that reduction of obesity would, in turn, lead to the reduction of risk factors as well.

Diabetes

Of all the benefits that come with RNYGB surgery, the improvement, or complete resolution of diabetes, could be one of the most pronounced. In a large meta-analysis of several studies, Shah et al.[168] found that the majority of all diabetic patients having surgery would see their condition completely resolved. One of the studies examined found that 32% of the surgery patients were taking medication for diabetes prior to surgery, while only 9% were still taking at the last observed follow-up.[110] Pories et al found that 83% of patients had recovery of diabetes after adequate follow-up.[149] In a study of 1,025 patients, Sugerman et al found that 86% of diabetics no longer had diabetes after a seven year follow-up.[184] In the SOS study after two years, 72% of diabetics had complete resolution.[173] In a compilation of these studies, as well as others, it was found that the average was 83.8% of diabetics saw complete resolution, while 90.6% saw resolution or improvement.[30] It is very clear that for morbidly obese diabetic patients, not only will the surgery benefit their mass, but chances are pretty high that it will resolve their diabetes.

Hyperlipidemia

While the diabetes data is very promising and dramatic, improvement in lipid levels after surgery is comparable, if not more promising. In the same compilation of studies, it was found that 94% of patients saw an improvement in hyperlipidemia, 95%

improvement in hypercholesterolemia, and 94.1% in hypertriglyceridemia.[30] In addition, the SOS study found that those with low HDL cholesterol saw that condition improve to normal ranges in 73% of patients.[173]

Hypertension

While the results are not as pronounced for hypertension, there still has been very well documented improvements with this condition after surgery as well. The Sugarman study found that 69%, 66%, and 51% of hypertensives saw resolution of their condition after 1, 5-7, and 10-12 years respectively.[184] The Buchwald et al meta-analysis found that 75.4% had resolution, while 87.1% saw improvement or resolution.[30] For some reason the SOS study had only 34% and 19% resolution at two and ten years post-surgery, respectively. However, when that result is compared to the 21% and 11% resolution rates of medically/lifestyle treated controls, the surgery still provides a benefit to the surgery.[173]

Sleep Apnea

Obstructive sleep apnea is quite common in the morbidly obese. As many as 77% of all patients seeking bariatric surgery have sleep apnea.[140, 201] This condition is often overlooked in the morbidly obese, but it has been shown that sleep apnea is linked to intermittent hypoxia, ventricular dysfunction, increased hospital stay, a risk factor for heart disease, as well as a risk for pulmonary complications after surgery.[16, 172, 220] The Buchwald meta-analysis found 86.6% resolution and 94.9% improved or resolved.[30] Another study found that even with a small amount of mass lost at one month post-

surgery significantly improved symptoms of sleep apnea. After nine months, none of the patients previously using CPAP (a machine used to treat sleep apnea) still required that therapy.[140]

Homocysteine

It is well understood that RNYGB surgery greatly improves several well-known factors for cardiovascular disease. With plasma homocysteine being an emerging, and potentially independent, risk factor for cardiovascular disease, it is important to study the effect that this surgery has on plasma homocysteine. Very few studies have examined plasma homocysteine levels after bypass surgery and those that have examined have been very inconclusive.

In a poorly designed retrospective study looking at all cardiovascular disease risk factors in 73 women after gastric banding surgery, researchers were surprised that plasma homocysteine levels were higher in the surgery compared to the control group (13.3 μM/L compared to 9.2 μM/L ($p<.001$)). There was no pre-surgical measurements for the index group and did not have any measure of the factors essential to plasma homocysteine metabolism such as B-12 and folic acid status.[94] In addition, the study was examining gastric banding which typically does not provoke as drastic of reduction in mass as RYNGB.

A better-designed, older study followed fifty three gastroplasty patients for one year following their surgery. The patients were predominantly female with an average BMI of 42.0 (±1.0). The patients were followed for a total of one year and lost an average of 32 kg (28% loss). This study measured folic acid and B-12 and accounted for

these vitamins in their analysis. They found that B-12 status did not change over the year, although information on B-12 supplementation was not described in the article. On the other hand, folic acid status decreased 20% during the year. Mean plasma homocysteine levels increased from 9.9 μM/L to 12.8 μM/L ($p<.0001$). Forty of fifty-three patients saw their plasma homocysteine increase. The authors assumed that this increase was directly related to folic acid status.[24] The big downfall of this study was the ambiguity of vitamin supplementation. In order to observe if changes in mass effect plasma homocysteine, vitamin status must be controlled.

A third study tracked 12 morbidly obese patients for a year after gastric banding. Mean weight loss was 32kg (26.7%) after twelve months. This study found that even when cobalamin and folic acid status remain constant, plasma homocysteine levels increased 10.2 to 12.1 μM/L ($p=.040$) at one year after gastroplasty. Surprisingly, the patients did not take any type of vitamin supplementation in this study, but somehow retained their vitamin status.[170]

On the contrary, a recent study has shown that a decrease in plasma homocysteine occurs after gastric bypass surgery. Patients (n=101, predominantly female) receiving gastric bypass surgery were followed for one year after their surgery. They were given 1000 μg/day of vitamin B-12. After one year, it was found that plasma homocysteine levels fell from 10.2 μM/L to 8.4 μM/L ($p<.0001$). Other than the mention of the B-12 supplementation, vitamin status was not addressed.[217]

With such contradicting findings, it is apparent that the literature is inconclusive about the impacts of bariatric surgery on plasma homocysteine. Some reasons for these limitations and discrepancies are that the first three studies were examining different

types of surgery. Some studies were poorly designed and failed to examine key components of plasma homocysteine metabolism.

Very little is known about the specific effects RNYGB surgery and its respective loss in mass has on the cardiovascular disease risk factor plasma homocysteine. With such a large proportion of the American population being obese and the high prevalence of cardiovascular disease, it is important to examine if the surgery alters serum homocysteine levels.

SPECIFIC AIMS OF THE STUDY

The primary aim of this study was to examine the change in serum homocysteine levels following laparoscopic Roux-en-Y gastric bypass surgery in morbidly obese patients. A secondary aim was to study the associations between concentrations of homocysteine at baseline and 12 months following surgery, with known determinants of its metabolism, including vitamin B-12 and folic acid, as well as anthropometric measures (body mass and body mass index) at the respective time points. Additionally, correlations were determined between changes during a one-year follow-up after bypass surgery in concentrations of homocysteine, vitamin B-12, and folic acid, as well as body mass, and body mass index .

HYPOTHESES

Based upon the research presented in the literature review, the hypothesis for the primary aim is that serum homocysteine concentrations will decrease following the Roux-en-Y surgery. For the secondary aims, serum homocysteine at baseline and 12

months post-surgery will be negatively related to vitamin status with lower homocysteine associated with higher vitamin levels at the respective time points. Changes in homocysteine will be negatively related to total mass/BMI loss with decreased homocysteine associated with greater mass/BMI loss. Changes in homocysteine will also be negatively correlated to changes in vitamin status. The more vitamin B-12 and folic acid increase, the more homocysteine will decrease.

METHODS

The current analyses is an ancillary substudy to an investigation that was conducted from 2005-2007. The original study was named the Laparoscopic Obesity Surgery Intensive Treatment (LOSE-IT) study. The primary purpose of LOSE-IT was to observe the changes of bariatric surgery (Laparoscopic Roux-en-Y gastric bypass) on physical function measures. Methods for LOSE-IT are described in this section. The current substudy used serum samples and data collected from the original study to examine the changes resulting from the surgery on homocysteine, vitamin B-12, and folic acid.

RECRUITMENT AND ELIGIBILITY REQUIREMENTS

Recruitment for the original study took place at Wake Forest University Baptist Medical Center through the general surgery office of Dr. Adolfo Fernandez. Patients were scheduled for surgery prior to learning about and being recruited for the study. Dr. Fernandez and his staff (Susan Butler, R.N.) assessed the patient's eligibility at an early screening visit for the surgery.

Men and women were eligible for the study if they had a BMI ≥ 40.0 kg/m^2 or ≥ 35.0 kg/m^2 with an obesity related comorbidity, such as hypertension, dyslipidemia, or diabetes. Since the primary purpose of the original study was to look at the effect of RNYGB on physical function, an additional inclusion criteria was that the patients have a self-reported difficulty in performing at least one of the following activities: lifting/carrying groceries, walking one-quarter of a mile, getting in and out of a chair, and climbing up or down stairs. They were also to be sedentary with no more than twenty minutes of exercise on two or fewer days per week. Since computed tomography (CT) scans were taken in the larger trial to assess regional body composition, an exclusion criteria was pregnancy at any point of the baseline or follow-up testing.

INFORMED CONSENT

If the surgery patient met the above eligibility requirements, Dr. Fernandez and/or his staff informed the patient of the study and administered the informed consent. The Institutional Review Board at Wake Forest University approved the study. Participants were alerted of potential risks associated with participating in the study. They were encouraged to contact the project coordinator if they experienced any negative effects of the testing procedures. The risk of the testing procedures was very minimal. Blood drawn at each visit might cause slight discomfort, bleeding and bruising, with minimal risk for infection and fainting.

STUDY PROCEDURES AND DATA COLLECTION

A total of twenty-eight participants were recruited and consented to the original study. Clinic staff obtained health history, current medications, and demographic information for all patients. There were two baseline appointments for each participant. Each baseline appointment occurred between four and twenty four days prior to receiving gastric bypass surgery. The average time between baseline measures and surgery was 7.7 days. Testing occurred at the Geriatric Research Center (GRC) located at Wake Forest University Baptist Medical Center (WFUBMC). A twelve-hour fasting blood sample was obtained at this time for measures of lipids, hormones, vitamin measurements, homocysteine, and inflammatory cytokines. This blood sample was stored as serum at -70 degrees Celsius. Participant also reported to the Geriatric General Clinical Research Center (GGCRC) during this visit. Using standard techniques, the GGCRC staff obtained baseline measures of blood pressure, body composition, height, and mass. Both height and mass were obtained with shoes and outer garments removed. Body mass index was then calculated from these measures. Body composition (fat mass and lean body mass), in both absolute and relative amounts, was measured using bioelectrical impedance assessment (BIA) (RJL Quantum II Desktop, Clinton Township, Michigan) with participants in a supine position. The GGCRC nutritionist also met with the participant to give instructions on completing dietary records. Four-days of dietary intake were recorded over a two-week period. Nutrient analyses for these records were determined using Nutrient Data System (NDS) software.

Participants underwent laparoscopic Roux-en-Y gastric bypass surgery. Surgery was performed by Dr. Fernandez using standard Roux-en-Y procedures for all surgeries. For a complete description of the surgical procedures, see Higa, 2001.[85]

Follow-up assessments were conducted at three weeks, three months, six months, and one year post surgery. The follow-up appointments consisted of all measures obtained at baseline. Food diaries were mailed to the participants prior to each follow-up visit for completion. The participants returned these completed diaries at their scheduled appointment.

NUTRITIONAL SUPPLEMENTATION

As part of standard post-surgery care to prevent nutritional insufficiencies, participants were prescribed a regimen of nutritional supplements. Patients were told to take a daily multivitamin and mineral supplement, as well as a calcium supplement (600 mg) with vitamin D twice daily, and a daily Vitamin B12 supplement of 500 μg. If the participant did not respond well to the B-12 supplement, they received a monthly intramuscular injection of 1000 μg of B-12. Iron supplements were not routinely required, unless the patient had post-operative anemia. The multi-vitamin was in a chewable form and was used twice daily for the first month post-surgery, but after one month the patient converted to a regular multi-vitamin. Supplementation compliance was not monitored. However, blood concentrations of vitamin B-12 and folate were assessed.

HOMOCYSTEINE MEASUREMENT

Serum samples from each participant's five visits were analyzed in the the lab of Carolina Liquid Chemistries (CLC) (Winston-Salem, NC) which is located in the Wake Forest University Research Park. The CLC Biolis 24i chemistry analyzer was used to perform these assays. This analyzer used a recombinant enzymatic cycling assay (RECA). The analyzer used a reaction of homocysteine and L-serine to form cystathionine catalyzed by cystathionine synthase. This reaction was followed by the conversion of cystathionine to homocysteine, pyruvate, and ammonia catalyzed by cystathionine β-lyase (CBL). The rate of pyruvate production was measured by inclusion of lactate dehydrogenase and nicotinamide adenine dinucleotide and was directly proportional to the concentration of homocysteine. (Carolina Liquid Chemistries, HCY test kit literature)

The analytical range for this assay is 0.00-86.6 μM/L. No sample exceeded this range so no dilutions were necessary. The reported sensitivity for the assay is 0.0 μM/L. Twenty replicates in two different levels (high and low) were run to confirm precision. The standard deviation for these levels was .24 and .27 μM/L which had a CV% of 3.3% and 0.8% respectively.

FOLIC ACID MEASURMENTS

Folic acid assays were preformed in the gerontology laboratory of Dr. Barbara Nicklas, located in the Nutrition building at Wake Forest University Baptist Medical Center. This assay was run using the IMMULITE 1000 analyzer. Folic acid was a boil, competitive, liquid-phase, ligand-labeled, protein-binding chemiluminescent assay. It used *in situ* immobilization and contained an anti-ligand detection system. A polystyrene

bead, which was enclosed in the IMMULITE test units, was covered in a murine monoclonal antibody specific for folic acid binding proteins. (IMMULITE Folic Acid test kit literature)

The reference range for the assay was 3.00-17.00 ng/mL. The analytical range was 1.00-24.00 ng/mL. If a sample exceeded these ranges, it was diluted and analyzed again. The calculated sensitivity of the assay using six replicates was 0.00 ng/mL and the test claim sensitivity was 0.80 ng/mL. These measurements were well below 1.60 ng/mL, which was deemed the acceptable limit. Reportable range verification was run at four different levels with a minimum of three trials per level (six at level one). The %CV for each level never exceeded 2.52% which fell within acceptable limits. Ten more samples were run in three additional levels to confirm precision and accuracy. The CV% for each of those levels was 2.75%, 2.22%, and 2.08%, all of which were within acceptable limits.

VITAMIN B-12 MEASUREMENTS

Vitamin B-12 assays were also preformed in the gerontology lab at WFUBMC using the IMMULITE 1000 analyzer. Similar to folic acid, the vitamin B-12 assay is a solid-phase, competitive chemiluminescent enzyme immunoassay. This assay involved a preliminary heat denaturation step to release the vitamin B-12 from its carrier protein. The sample was then treated with hog intrinsic factor and introduced to a polystyrene bead coated with a B12 analog. (IMMULITE B12 test kit literature)

The reference range for the assay was 174.00-878.00 pg/mL. The analytical range was 150.00-1,000.00 pg/mL. If a sample exceeded these ranges, it was diluted and analyzed again. The calculated sensitivity of the assay using six replicates was 42.22

pg/mL and the test claim sensitivity was 125.00 pg/mL. 250.00 pg/mL was deemed the acceptable limit. Reportable range verification was run at four different levels with a minimum of three trials per level (six at level one). The %CV for each level never exceeded 6.4% which fell within acceptable limits. Ten more samples were run in three additional levels to confirm precision and accuracy. The CV% for each of those levels was 8.13%, 4.91%, and 3.80%, all of which were within acceptable limits.

DROP OUTS

A total of 28 participants were recruited for the original study. One participant opted out of the surgery after baseline data were collected. Two patients dropped out of the study due to complications resulting from the surgery. An additional four participants dropped out as they were no longer being followed by the surgeon or personal reasons (lack of time and no child care).

These dropouts led to a total of twenty participants with serum collected from all visits. Since only one of these participants was male, that patient was excluded to only examine females.

STATISTICAL ANALYSIS

All data were analyzed for normality through descriptive statistics and frequency histograms. Since serum homocysteine levels and vitamin B-12 were skewed, the data were transformed using the natural log. While all analyses were done using the log transformed data for both variables, raw data were reported for ease in understanding. Scatterplots for demonstrating correlational data are shown with the transformed data. Descriptive statistics, including means, ranges, and standard deviations were calculated

for homocysteine, both vitamins, body mass, BMI, as well as several dietary intake variables. One-way repeated measure ANOVA determined if the surgery altered variables associated with the aims of this study. If a significant time interaction was found, LSD was used as the Post-Hoc test for variables that showed differences across time. Pearson product correlations were used to determine if significant relationships existed between the variables. A p value of < 0.05 was deemed to be statistically signficiant.

RESULTS

Data for nineteen female patients were analyzed for the current study. All subjects underwent the same surgical procedure by the same surgeon. Patient characteristics are displayed in Table 3. Mean age of participants was 45.7 (±8.8) years. Of the nineteen participants, seventeen were Caucasian and two were African American. At baseline, BMI ranged from 36.3 kg/m^2 to 65.9 kg/m^2 with a mean of 54.4 (±7.1) kg/m^2. Minimum and maximum measures of body mass at baseline were 98.3 kg to 193.9 kg with a mean and standard deviation of 147.2 (±24.0) kg.

TABLE 3.

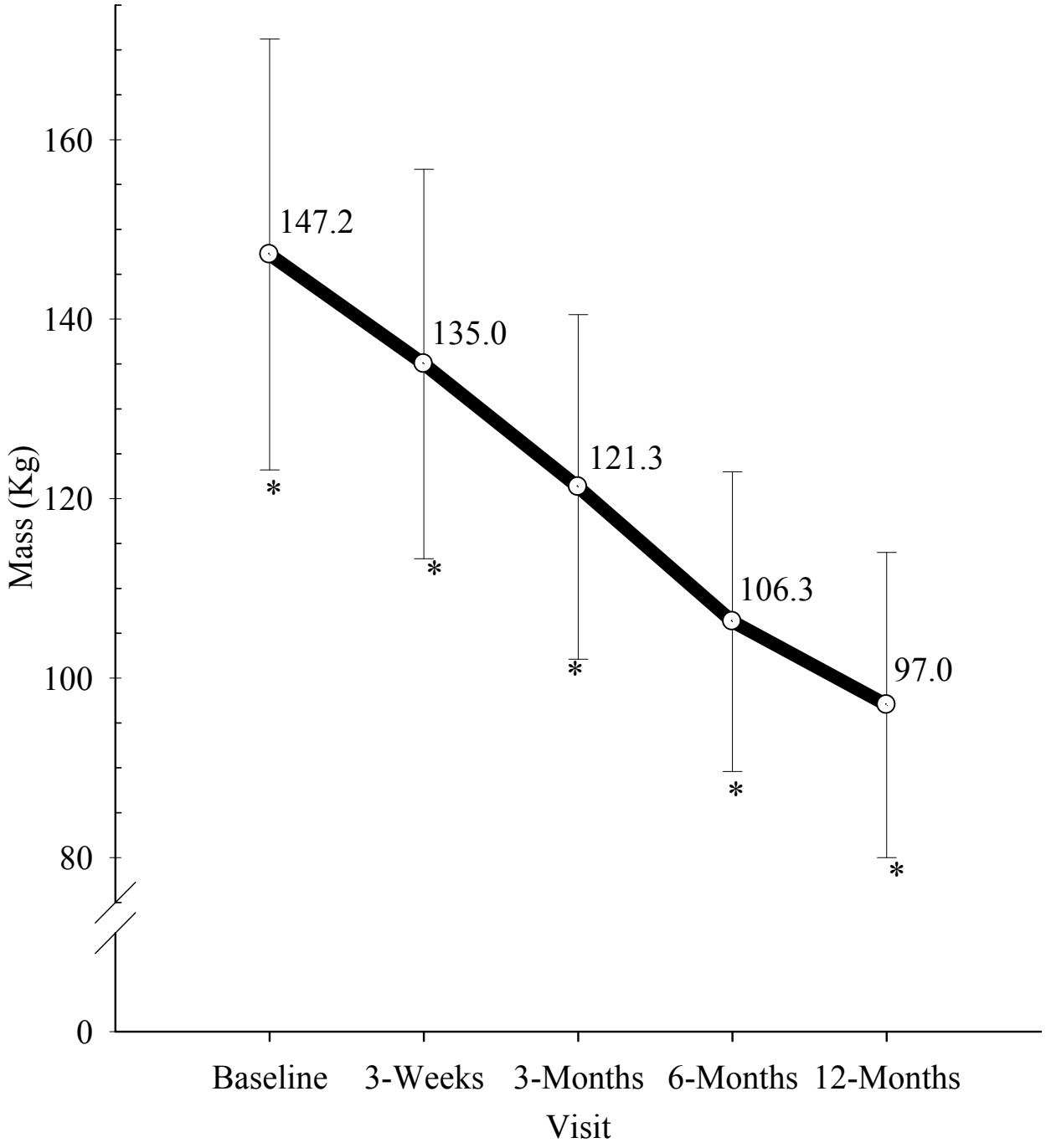
Baseline Patient Characteristics (mean, ±standard deviation):

Age (years)	45.4 (±8.8)
Body Mass (kg)	54.4 (±7.14)
BMI (kg/m^2)	147.2 (±24.0)
Comorbidities (mean number per patient)	4.6 (±1.6)
Comorbidities (% prevalent)	
Hypertension	63%
Hyperlipidemia	42%
CVD	0%
Diabetes	42%
Sleep Apnea	57%
Depression	57%
Medications (mean number used per patient)(% yes)CVD (% yes)	1.8 (±1.5)

Following the surgical procedure, body mass was determined at 3 weeks, 3 months, 6 months, and 12 months. As shown in Figure 2, body mass was significantly lower at each time point as compared to baseline, and at each subsequent time point compared to the previous time. Body mass was reduced from 147.2 (±24.0) kg at baseline to 135.0 (±21.6) kg at 3 weeks, 121.3 (±19.2) kg at 3 months, 106.3 (±16.2) kg at 6 months, and 97.0 (±17.0) kg after one year. Participants lost an average of 50.2 (±15.2) kg over the course of the study. This is also demonstrated with percent change in mass (Figure 3); percent change from baseline reached was 7.8 (± 1.5)% at 3 weeks, 18.0 (± 2.7)% at 3 months, 26.1 (± 4.0)% at 6 months and 33.9 (± 7.1)% at 12 months. Mean BMI was reduced by 18.5 (4.7) kg/m^2 at 12 months compared to baseline with a final follow-up value of 35.9 (±6.2) kg/m^2.

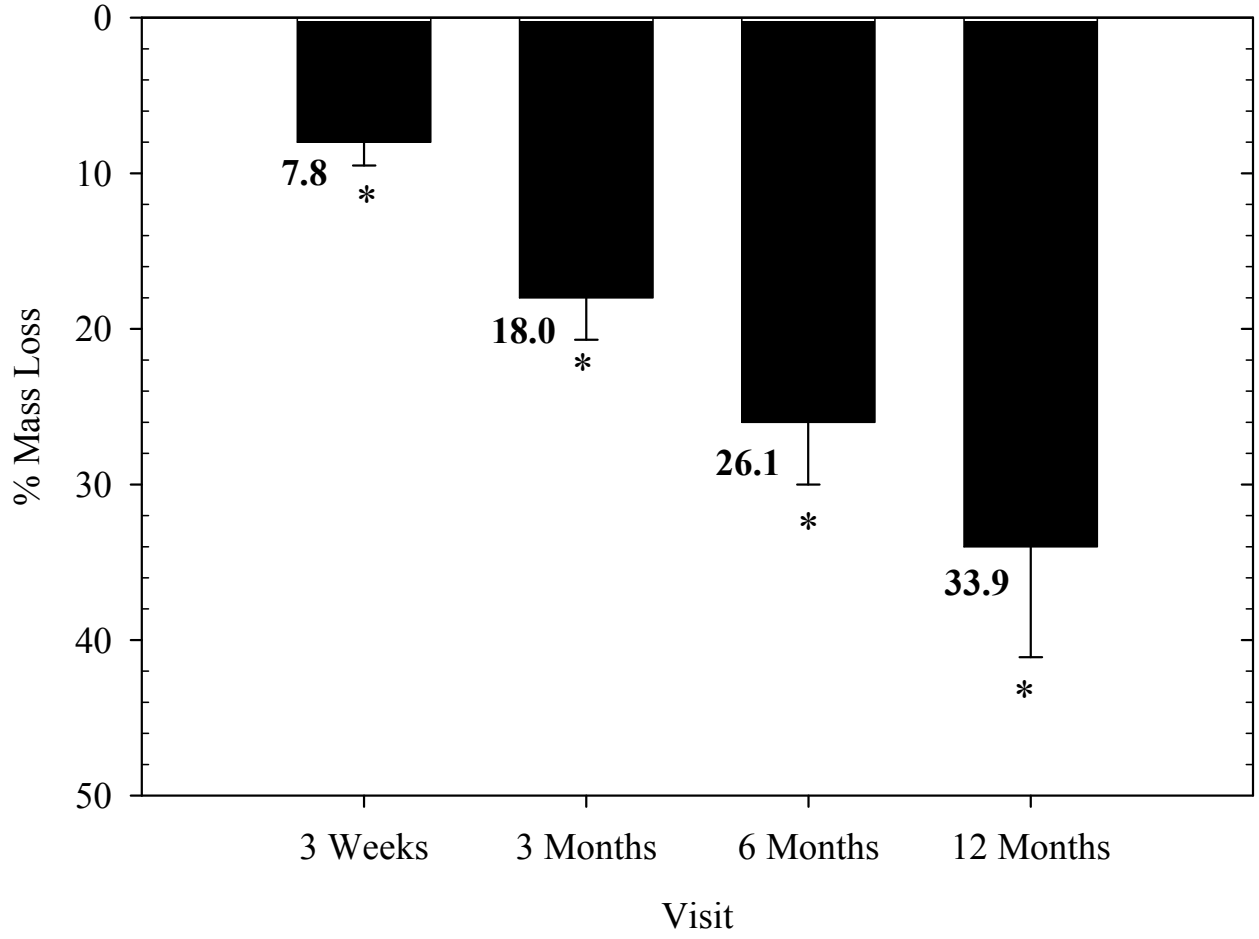
FIGURE 2.

Change in Mass Between Baseline and One Year Follow up



* indicates significantly different from all other visits

FIGURE 3.
Percent Mass Loss Between Baseline and Follow-Up Visits



* indicates significantly different from all other visits

HOMOCYSTEINE

Because homocysteine data were not normally distributed, these data were transformed using the natural log prior to comparison analysis. For ease in understanding, non-transformed serum homocysteine levels are used in the text and in Figures 4 and 5. Using repeated measures of variance analysis, no significant differences were observed across time ($p=0.879$). Baseline homocysteine levels ranged from 7.2 μM/L to 22.8 μM/L with a mean of 10.4 (±3.5) μM/L. Mean values for homocysteine at the follow-up time points were 11.7 (±3.9) μM/L at 3 weeks, 11.4 (±3.9) μM/L at 3 months, 11.6 (±2.9) μM/L at 6 months, and 10.3 (±2.4) μM/L at 12 months. Serum levels ranged from 6.6 μM/L to 15.7 μM/L at the conclusion of the study. To show individual responses, each participant's values for the five visits are illustrated in Figure 5. As shown, the majority of individuals had minimal changes with one participant having a tremendous drop across time with others having transient increases followed by reductions to baseline concentrations.

FIGURE 4.
Mean Serum Homocysteine Levels

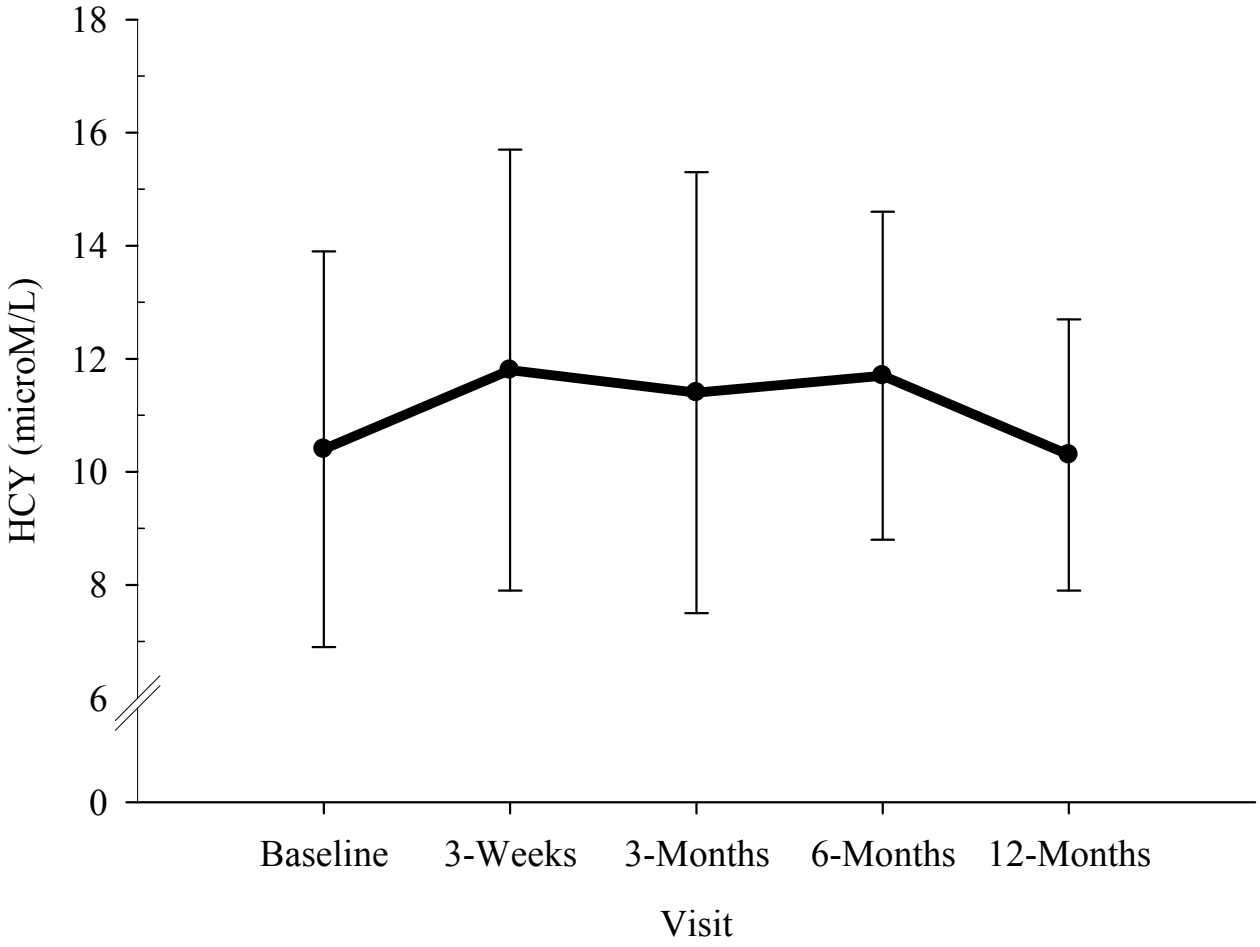
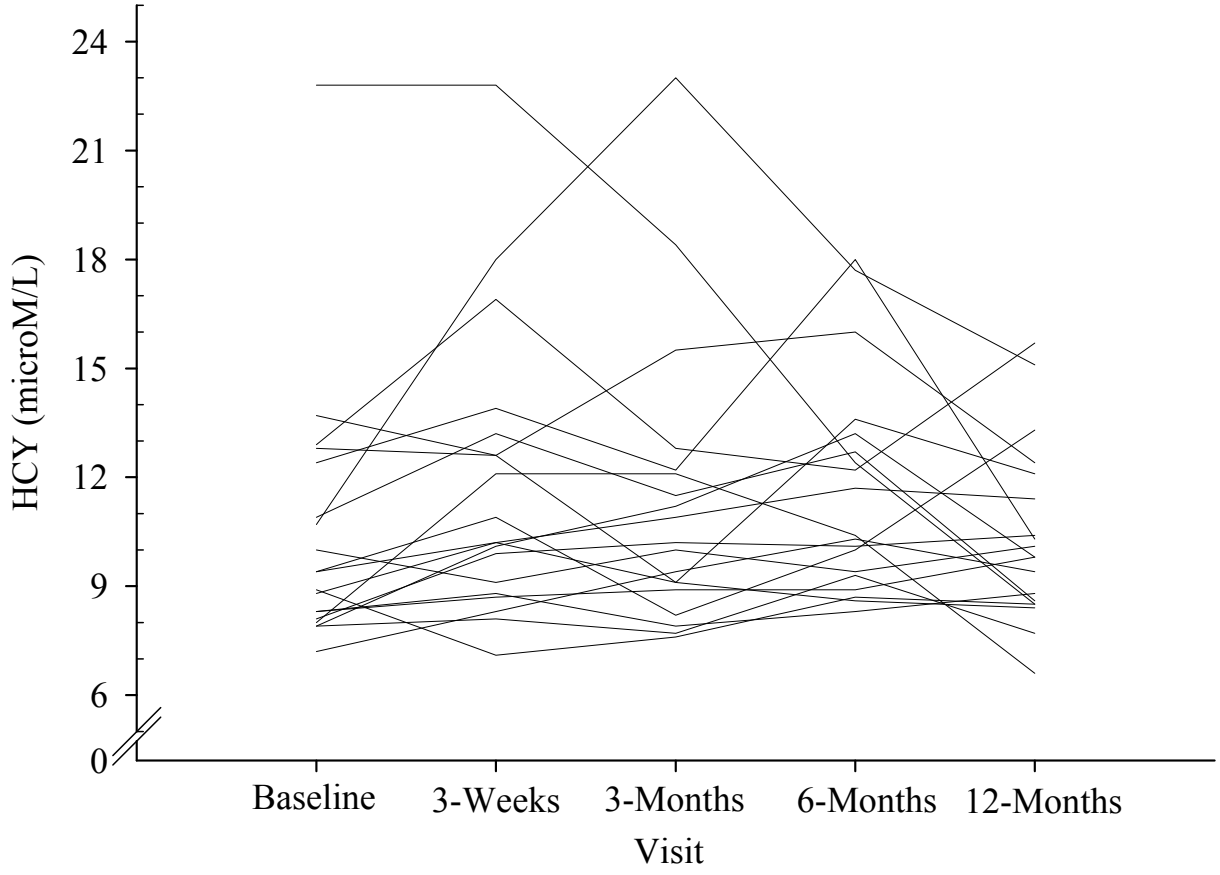


FIGURE 5
Individual Serum Homocysteine Levels



SERUM FOLIC ACID

Serum levels of folic acid were determined at baseline and each of the follow-up visits. Using repeated measures of variance analysis, no significant differences were observed over the course of twelve months (p=0.827) (Figure 6). At baseline, mean serum folic acid level was 24.6 (±11.8) ng/mL. Minimum serum level was 10.6 ng/ml while the maximum was 50.6 ng/ml. Mean values for folic acid at the follow-up time points were 26.5 (±10.1) ng//mL at 3 weeks, 23.1(±10.9) ng//mL at 3 months, 21.1 (±7.7) μM/L at 6 months, and 28.4 (±11.8) ng//mL at 12 months. Serum levels ranged from 11.8 ng/ml to 48.7 ng/ml at the conclusion of the study. To show individual responses, each participant's values for the five visits are illustrated in Figure 7.

FIGURE 6
Mean Serum Folic Acid Levels

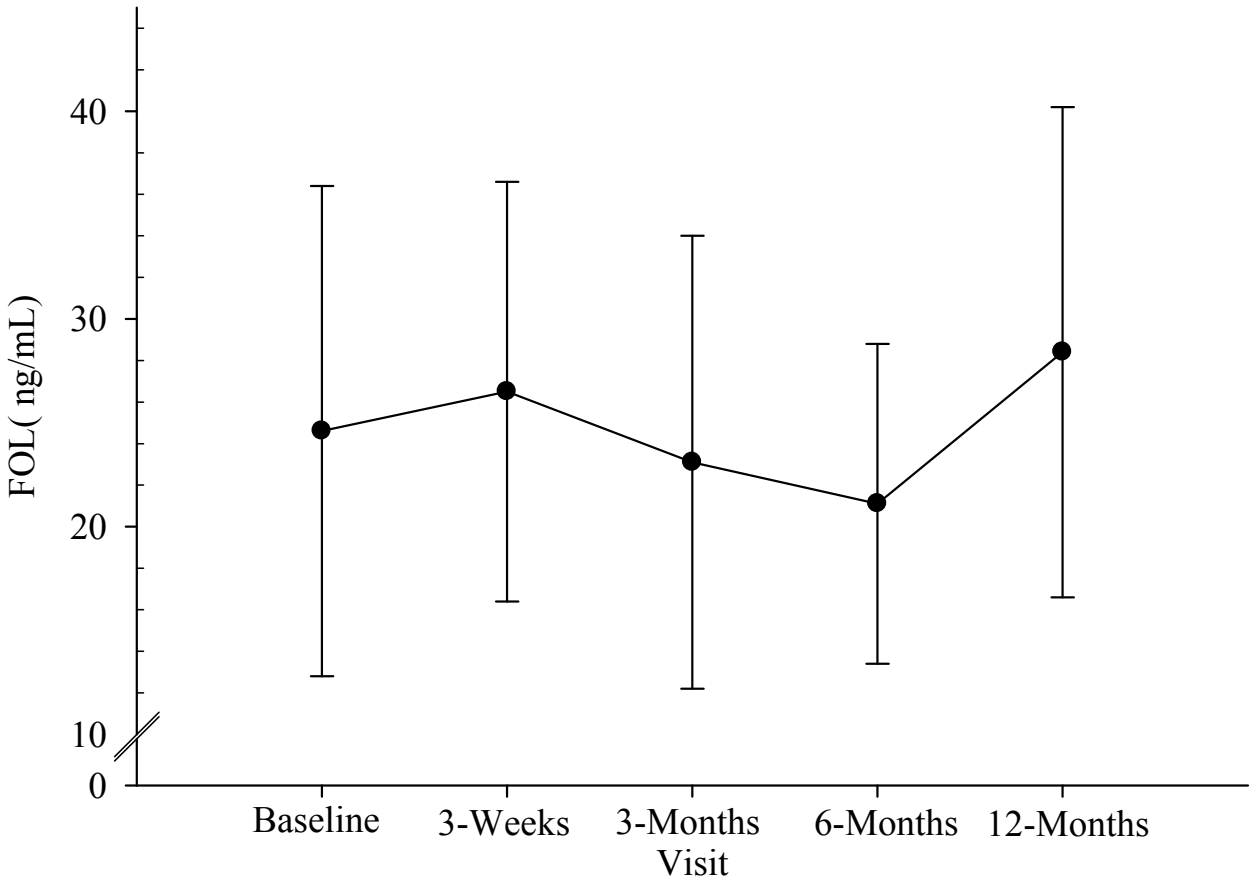
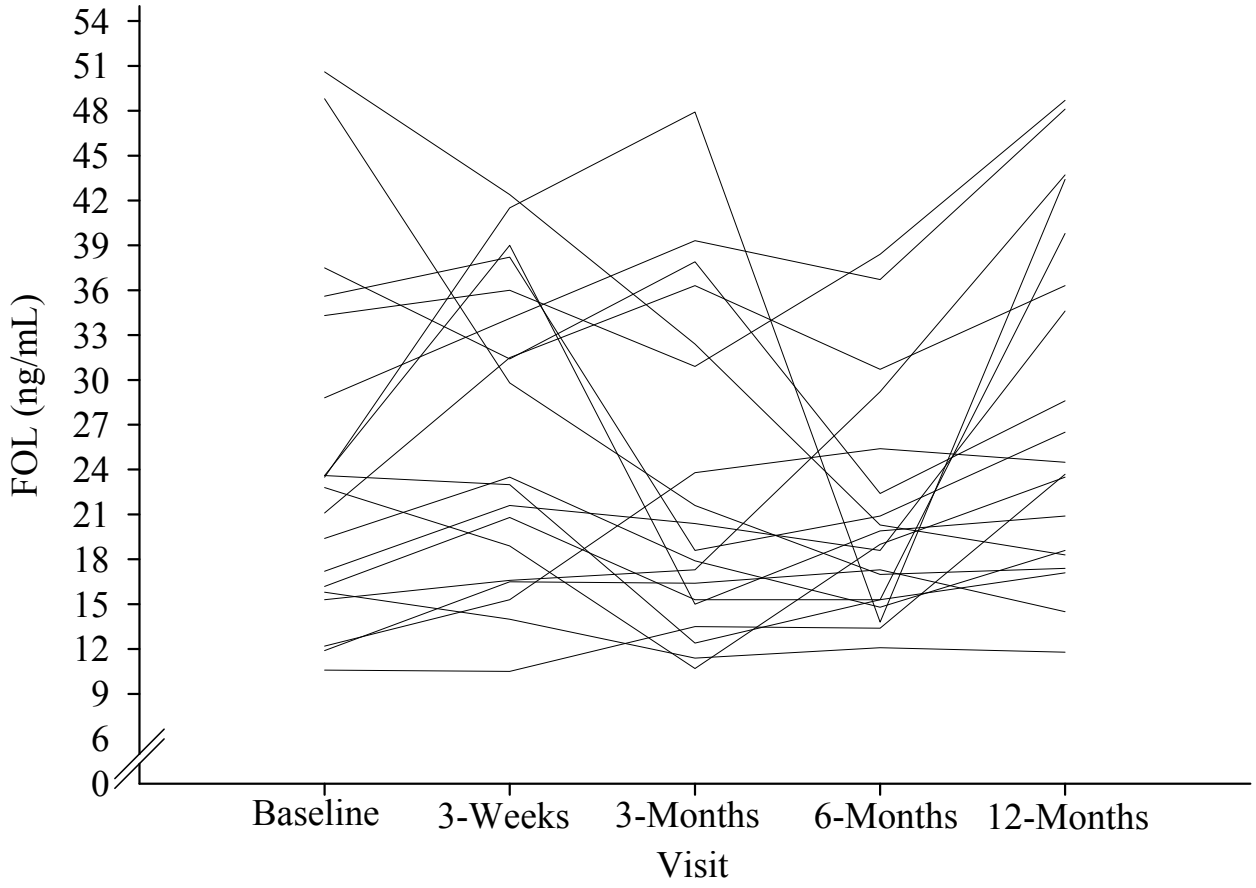


FIGURE 7
Individual Serum Folic Acid Levels



SERUM VITAMIN B-12

Because vitamin B-12 data were not normally distributed, these data were transformed using the natural log prior to comparison analysis. For ease in understanding, non-transformed vitamin B-12 levels are used throughout the text and in Figures 8 and 9. Using repeated measures of variance analysis, no significant differences were observed across time ($p=0.377$). At baseline, mean serum vitamin B-12 was 617.6 (±351.1) pg/ml. Minimum serum level was 253.0 pg/ml while the maximum was 1881.0 pg/ml. Mean values for vitamin B-12 at the follow-up time points were 1071.3 (±449.8) pg/ml at 3 weeks, 953.8 (±610.8) pg/ml at 3 months, 774.5 (±539.6) pg/ml at 6 months, and 749.8 (±766.6) pg/ml at 12 months. Serum levels ranged from 205.0 pg/ml to 3677.0 pg/ml at the conclusion of the study. To show individual responses, each participant's values for the five visits are illustrated in Figure 9.

FIGURE 8
Mean Serum Vitamin B-12 Levels

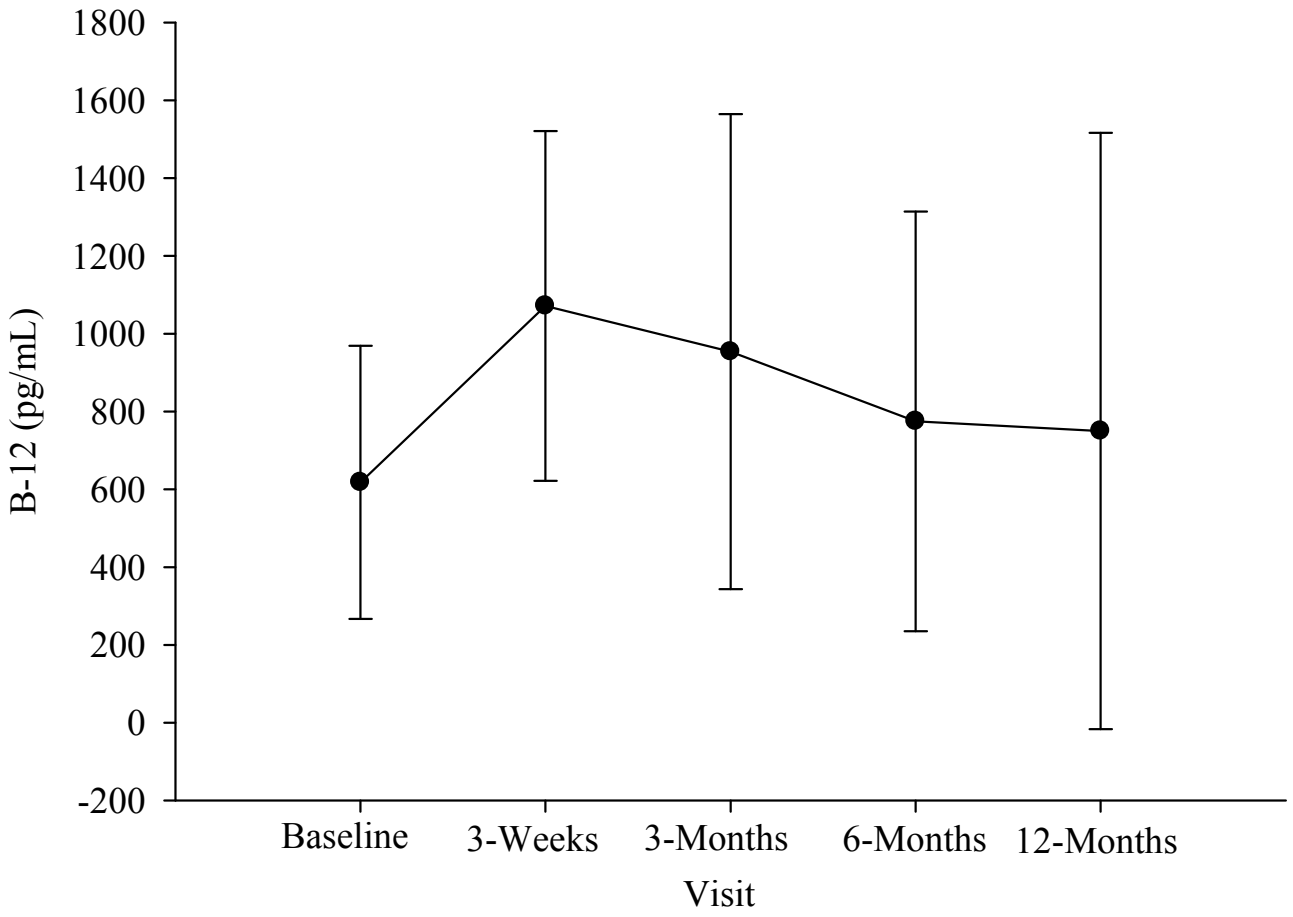
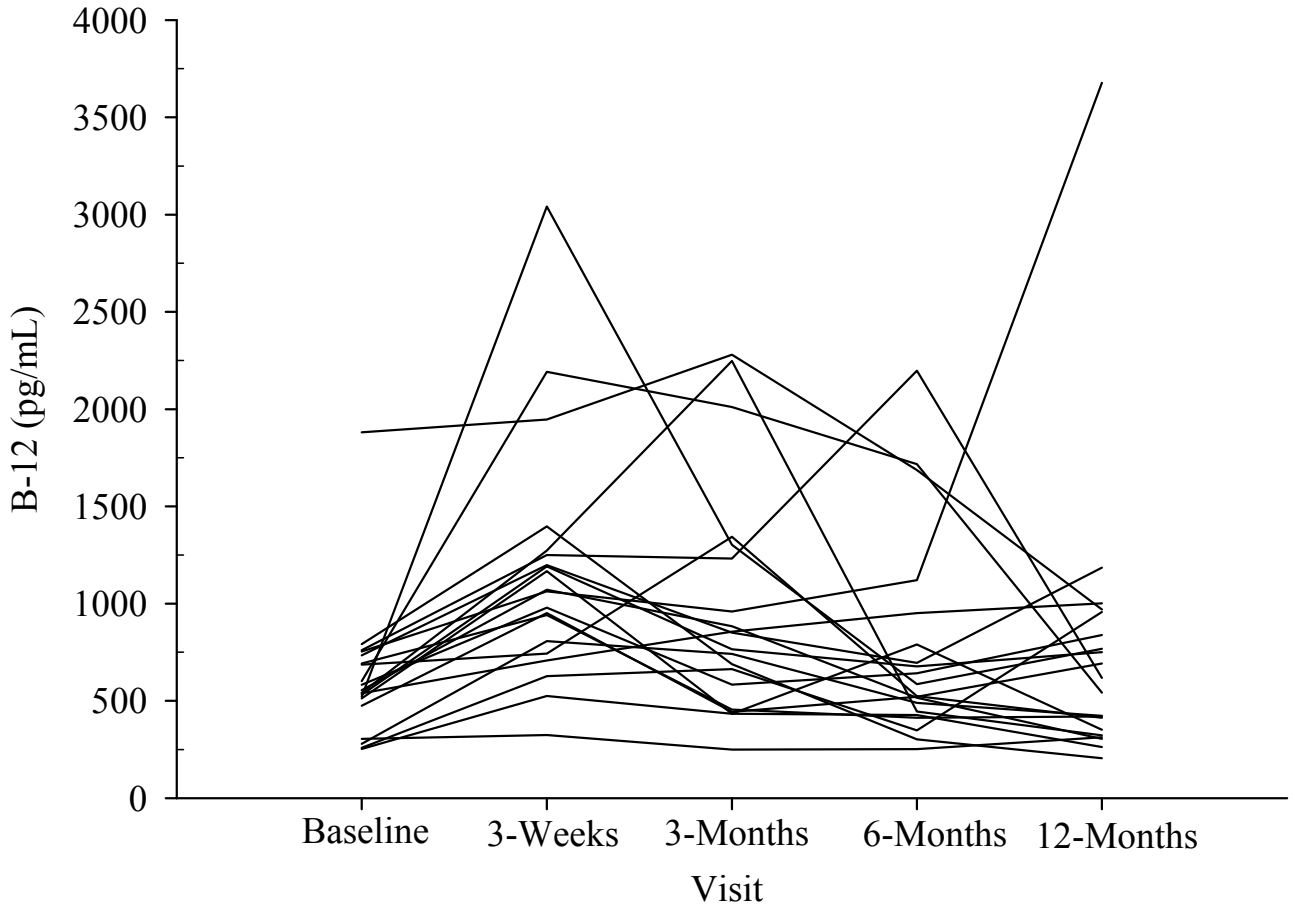


FIGURE 9
Individual Serum Vitamin B-12 Levels



DIETARY RESULTS

Results from the dietary logs are shown in Table 4. Nutrients that have been shown to affect homocysteine levels are presented. Energy intake was significantly less following the surgery as compared to baseline. Intake of protein, methionine, folic acid, vitamin B-12, alcohol, and caffeine did not significantly change during the course of the study.

TABLE 4.
Daily Nutrient Intake At Baseline and Follow-up Time Points (mean, ±S.D.)

	Baseline	3 weeks	3 months	6 months	12 Months	P-Value
Calories (kcal)	2186.4 (±657)	639.6 (±148)	821.1 (±281)	1009.3 (±284)	1202.6 (±552)	>.001
Protein (g)	91.3 (±22.5)	53.1 (±16.2)	58.6 (±24.3)	67.7 (±18.6)	68.8 (±26.0)	.314
Methionine (mg)	2.1 (±.52)	1.1 (±.31)	1.1 (±.41)	1.5 (±.42)	1.4 (±.56)	.239
Vitamin B-12 (μg)	6.1 (±3.7)	3.1 (±1.4)	2.7 (±2.7)	3.8 (±3.9)	4.4 (±2.8)	.377
Folic Acid (μg)	465.4 (±299.9)	132.2 (±60.3)	151.6 (±62.3)	193.5 (±93.1)	324.2 (±185.2)	.281
Alcohol (g)	.43 (±1.31)	.00 (±0.0)	.10 (±.39)	.19 (±.79)	.01 (±.008)	.385
Caffeine (mg)	57.2 (±42.8)	6.6 (±13.8)	19.7 (±26.8)	31.6 (±36.2)	79.86 (±117.5)	.179

CORRELATIONS

Pearson-Product Correlations were performed between baseline values of homocysteine and body mass, vitamin status, and other dietary factors that are known to influence homocysteine concentrations. Correlation coefficients and p values from these associations are shown in Table 5. Vitamins B-12 and folic acid were not significantly correlated ($p>0.05$) to homocysteine at baseline with p-values of .097 and .148, respectively. However, serum homocysteine was significantly correlated to serum B-12 and folic acid levels at the twelve month follow-up visit ($r= -.486$, $p=.041$ for B-12 and $r= -.522$, $p=.026$ for folic acid). None of the other dietary variables (protein, methionine, caffeine, and alcohol intake) were significantly correlated with homocysteine at either time period. The total change in serum homocysteine over the course of the study was significantly correlated to the overall change in serum B-12 ($r= -.624$, $p=.006$), serum folic acid ($r= -.511$. $p=.030$), and BMI reduction ($r= .580$, $p=.028$). For vitamin B-12 and folic acid, increased homocysteine levels were associated with decreased vitamin levels in the blood. Decreased BMI was associated with increased homocysteine. The scatter plot in Figure 10 displays the relationship between change in transformed homocysteine and BMI reduction. Figures 11 and 12 display scatter plots for change in Ln HCY and change in Folic Acid and change in Ln vitamin B-12, respectively.

TABLE 5.
Pearson-Product Correlations

	Baseline Ln Homocysteine	12 Month Ln Homocysteine	Δ Ln Homocysteine
Baseline BMI	r= -.275, p=.270		
Baseline Body Mass	r= -.292, p=.239		
Baseline Ln B-12	r = -.403, p=.097		
Baseline FOL	r = -.355, p=.148		
Baseline Alcohol	r = -.176, p=.546		
Baseline Caffeine	r = -.141, p=.631		
Baseline Methionine	r = -.468, p=.091		
12 Month BMI		r=.226, p=.367	
12 Month Body Mass		r=.150, p=.553	
12 Month Ln B-12		**r = -.486, p=.041**	
12 Month FOL		**r = -.522, p=.026**	
12 Month Alcohol		r = -.116, p=.692	
12 Month Caffeine		r = -.150, p=.608	
12 Month Methionine		r = .244, p=.400	
Δ Ln B-12			**r = -.624, p=.006**
Δ FOL			**r = -.511. p=.030**
Δ Body Mass			r = .460, p=.055
Δ BMI			**r = .580, p=.028**
Δ Alcohol			r = -.308, p=.285
Δ Caffeine			r = -.241, p=.406
Δ Methionine			r = -.259, p=.372

Figure 10
Correlation Between Δ Ln HCY and BMI Reduction
r = .580, p=.028

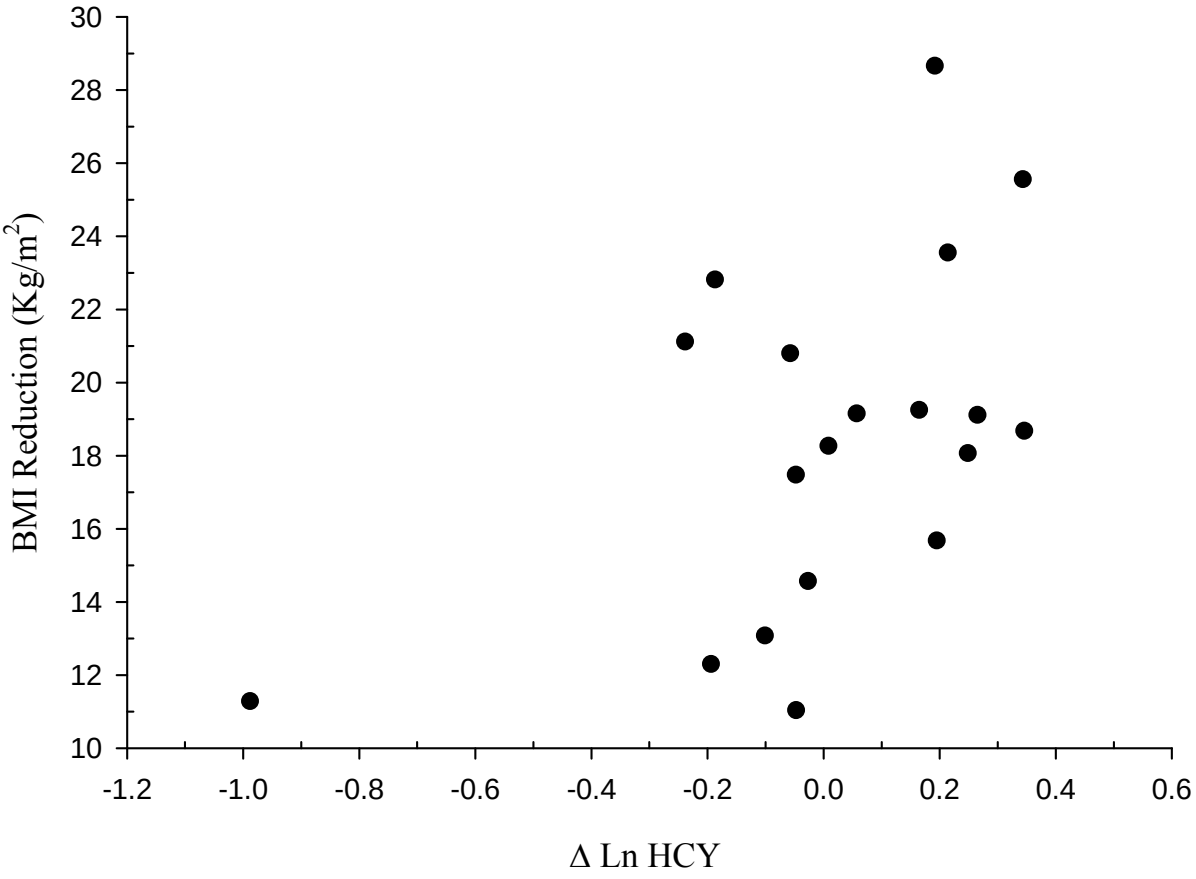


FIGURE 11

Correlation Between Δ HCY and Δ B-12

r = -.624, p=.006

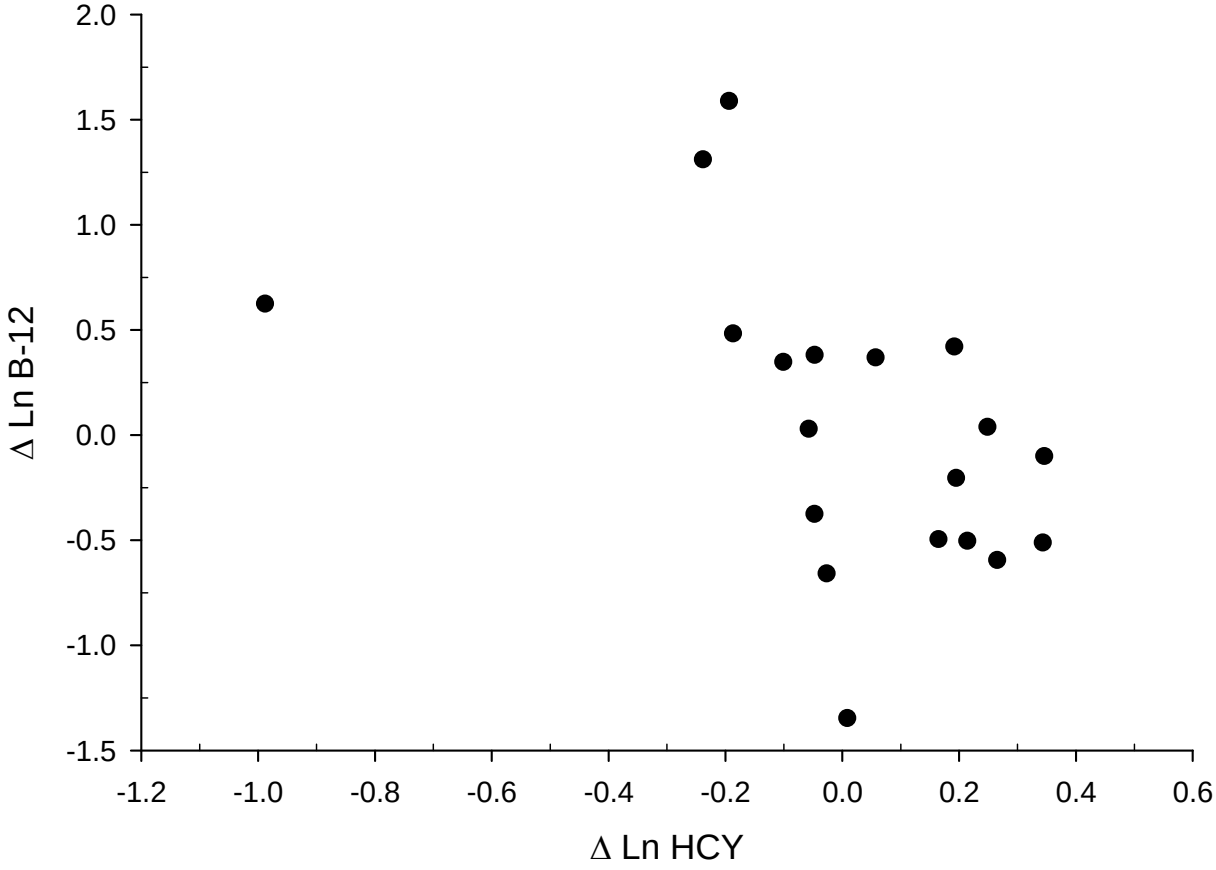
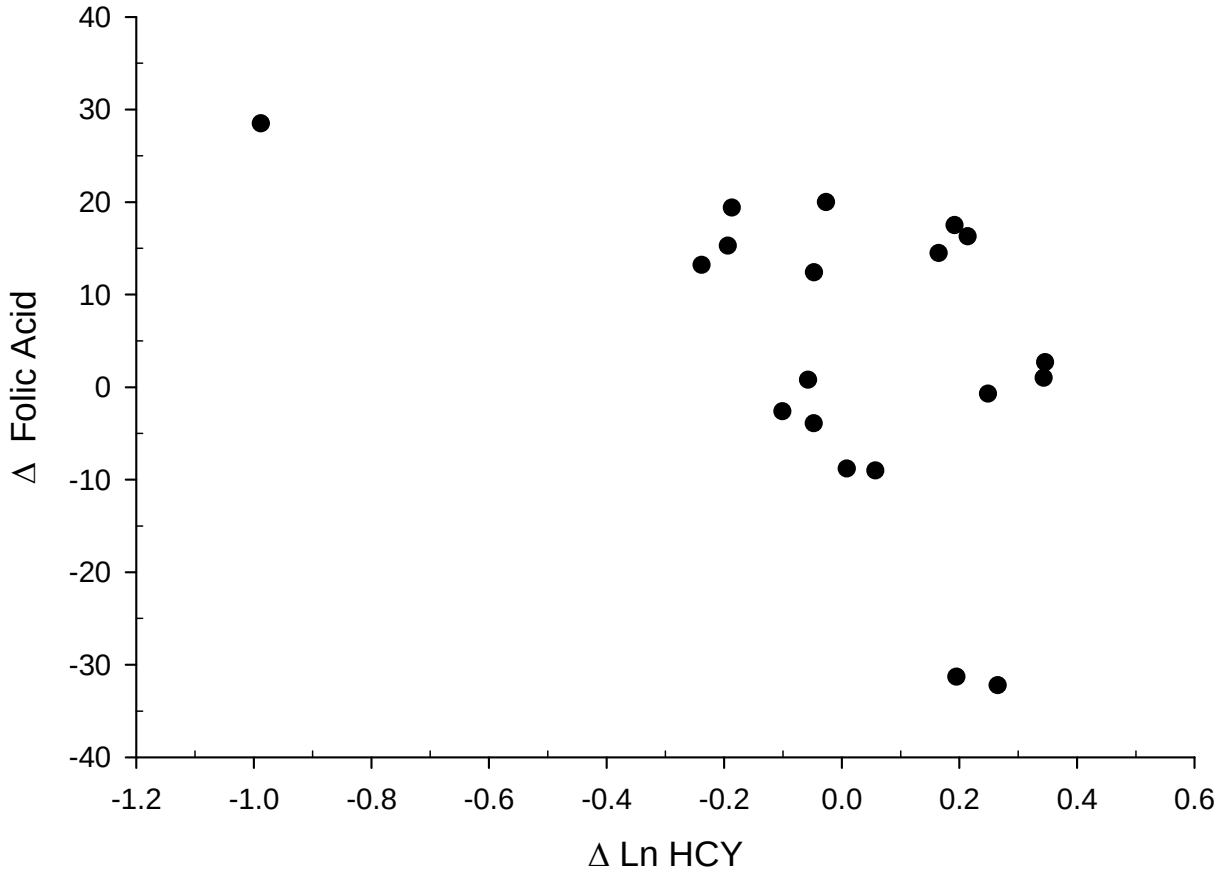


FIGURE 12

Correlation Between Δ Ln HCY and Δ Folic Acid

r = -.511. p=.030



DISCUSSION

The primary aim of this study was to examine the change in serum homocysteine levels following laparoscopic Roux-en-Y gastric bypass surgery in morbidly obese patients. A secondary aim was to study the associations between concentrations of homocysteine at baseline and 12 months following surgery, with vitamin B-12 and folic acid, as well as anthropometric measures (body mass and body mass index) at the respective time points. Because of the proven reduction in other cardiovascular disease risk factors post surgery, as well as the prophylactic regimen of vitamin supplementation that was prescribed to our subjects, we hypothesized that serum homocysteine would decrease following surgery. A recent study also found surgery patients given a large daily supplement of B-12 had decreased plasma homocysteine levels post gastric bypass surgery. [217] Because vitamin B-12 and folic acid are involved in the metabolism of homocysteine in the cell, we also hypothesized that serum homocysteine would be negatively correlated with blood levels of vitamins B-12 and folic acid, with higher levels of vitamins associated with lower concentrations of homocysteine.

Our results do not support our first hypothesis as homocysteine was not significantly changed from baseline to twelve months post surgery (p=.879). Whereas baseline values of homocysteine and folic acid and vitamin B-12 were not correlated, there was a significant negative correlation between these markers at 12 months. These latter correlations were expected as both folic acid and vitamin B-12 are involved in the

metabolism of homocysteine to methionine. Initially, it was surprising that correlations were not apparent at baseline. However, upon further consideration, this lack of association may be because at adequate levels of the vitamins, further reduction in homocysteine is not going to be apparent. In other words, when B-12 and folic acid reach a certain level, they may no longer influence homocysteine metabolism. It may be that these vitamins only influence homocysteine levels when there is a shortage. As the participants of this study were not considered deficient of either vitamin during the study, it is not surprising that vitamin B-12 and folic acid were not found to be correlated with homocysteine at most visits. Additionally, these data may indicate that other factors are playing a role in homocysteine metabolism in this popuatlion. This may be related to being in a condition of great metabolic fluctuations with tremendous reduction in dietary intake (< 1,000 kcals per day for the first 6 months after surgery) and subsequent loss of body weight. Although purely speculative, the dramatic loss in mass participants experienced in initial months might have interfered with an aspect of the methionine or folic acid cycles and affected the normal relationship between the vitamins and homocysteine. Between visits at six and twelve months, mass loss was less dramatic than between other visits and might partially explain why serum homocysteine correlated stronger with serum vitamin B-12 and folic acid at twelve months.

While homocysteine, vitamin B-12, and Folate did not significantly change over the course of the study, the individual responces were found to be negatively correlated between change values. In other words, the more a participant's vitamin status increased, the more their homocysteine levls decreased (Figures 11 and 12).

As extreme obesity (BMI $\geq$ 40.0 kg/m^2) has rapidly become more prevalent in America, there has been a considerable rise in the number of patients having gastric bypass surgery as treatment.[180] While surgical treatment is considered successful with over a 30% loss in body mass observed[168], there are potential hazards, namely impaired nutrient absorption that accompanies malabsorptive procedures, including RNYGB. Many nutrients are preferentially absorbed in the proximal portion of the small intestine. As this portion of the small intestine is bypassed in RNYGB, it is not surprising that vitamin deficiencies are common following surgery. [168] Few studies have examined plasma homocysteine levels after bypass surgery and those that have are inconclusive with limitations on interpreting their findings. The primary results from several of these earlier works show an increase in plasma homocysteine levels post surgery. [24,53, 94, 170] However, three studies did not appropriately address vitamin status in their analysis. [24, 94,170] In contrast, a more recent study using vitamin supplementation found plasma homocysteine levels decreased from 10.2 μM/L to 8.4 μM/L one year post surgery. [217]

As stated, vitamins B-12 and folic acid are well-known predictors of plasma homocysteine levels and if deficiency in one or more of these nutrients occurs, it will likely increase homocysteine levels. Failure to measure these crucial vitamins is a confounding factor in interpreting the homocysteine data in the three studies. Dixon et al [53] found higher homocysteine in 293 patients after Lap-Band surgery (10.4 μM/L increased to 11.0 μM/L). However, they did not find changes in plasma vitamin B-12 over the course of the study (376 pg/mL at baseline and 365 pg/mL at follow-up). Furthermore, plasma folic acid was found to be no different one year post surgery (8.4 ng/mL to 8.6 ng/mL). These findings challenge the notion that an increase in plasma

homocysteine after surgery is directly attributable to vitamin deficiencies. Following the surgery, Dixon et al report that patients were recommended to supplement their diet with a vitamin supplementation. Researchers found that when comparing patients who took the recommended supplements to those who did not, the non-users had significantly higher plasma homocysteine levels. The authors suggested that when undergoing mass loss, patients had an altered dose-response relationship between plasma homocysteine and vitamins B-12 and folic acid.[53] It appears as if serum levels of vitamins B-12 and folic acid levels are important but may not completely control serum homocysteine levels. It is possible that other factors seem to alter homocysteine levels post-surgery. In the current study, we examined serum vitamin B-12 and folic acid levels in addition to homocysteine in an attempt to gain a better understanding of their relationships.

Only one participant had a homocysteine level at baseline that would be classified as hyperhomocysteinemic (22.8 μM/L), using the most commonly cutoff of 15 μM/L [8, 75, 142, 152, 191, 192, 197]. If a lower reference range of 12 μM/L had been used, five participants would have been classified as hyperhomocysteinemic at baseline. The distribution curve at baseline exhibited the characteristic positive skew that has been described in the literature review. At the twelve month follow-up visit, two participants were hyperhomocysteinemic with serum levels above 15 μM/L and five participants had serum levels over 12 μM/L. The distribution curve at this time point also exhibited a positive skew. Both serum folic acid and serum vitamin B-12 status were in normal ranges for all patients at baseline. At no time point during the study were any of the patients considered deficient in either of the nutrients. Folic acid deficiency is normally defined by any serum level less than 3 ng/mL. The lowest baseline serum level was 10.6 ng/ml. Folic acid

levels remained relatively consistent throughout the course of the study. Average serum folic acid levels at twelve months were well above the minimum of 3 ng/mL at 28.4 ng/ml (±11.8). The lowest serum level of folic acid at follow up was 11.8 ng/mL. During the course of the study, no participant fell below 10 ng/ml of serum folic acid. Using a serum level of 150-200 pg/ml as the criteria for vitamin B-12 deficiency no patients were considered deficient and only three participants had serum levels less than 300 pg/ml at baseline. One participant was close to the 200 pg/ml cutoff at 205.0 pg/ml at the end of the study. Only two participants had follow-up serum levels below 300 pg/ml. At no point during the study did any patient have serum vitamin B-12 levels below 200 pg/ml.

As there have been reports in the literature that various dietary variables can influence homocysteine, several of these variables were assessed in our analysis. Methionine levels were not significantly correlated with homocysteine during this study. The literature shows that methionine intake usually does not influence fasting plasma homocysteine levels even though this sulfur containing amino acid is the immediate precursor to homocysteine in the metabolic pathway. [10, 171,200] Since participants in this study were in a fasted state during each blood draw, the results of this study appear to confirm this finding. Since methionine is derived from dietary protein, as expected, no significant correlations between protein consumption and homocysteine were found.

Both alcohol and caffeine have been shown to be strong determinants of plasma homocysteine levels. In this study, neither substance was correlated with homocysteine. Most of the literature consistently reports that these substances only influence homocysteine levels in high quantities. Between 4 and 6 cups of coffee per day have been shown to raise homocysteine levels while moderate (<4 cups) had no effect. [136,182]

Alcohol has a similar effect with only high levels of consumption having an effect on plasma homocysteine. [68] The intake of caffeine and alcohol observed in this study were moderate to none, therefore it was not surprising there were no significant correlations with these variables.

Another interesting relationship observed in this study is between change of serum homocysteine levels and change in BMI and mass. We hypothesized that a decrease in mass would be correlated with a corresponding decrease in homocysteine. The data from this study indicate the opposite. Significant correlations were apparent between change of serum homocysteine and changes in BMI and mass. The loss of more body mass/BMI was related to greater increases in serum homocysteine levels (Figure 10).

A possible explanation for the relationship between BMI/body mass loss and increased serum homocysteine levels might be attributable to changes in lean body mass. It has been shown that patients receiving gastric bypass not only lose fat-mass, but in most cases will lose a considerable amount of lean body mass as well. The loss in lean body mass might affect serum homocysteine in multiple ways. Primarily, fat mass loss far exceeds that of lean body mass loss post-surgery. Carey et al found that after six months, fat mass accounted for 66.5% of the total mass lost, while LBM loss was responsible for the other 33.5%.[33] Wadstrom et al found similar results but also calculated LBM to fat mass ratios at each time from post surgery for a year. They found roughly a 1:1 ratio at baseline, but a 1.58 ratio after one year.[206] These findings suggest that while lean body mass is lost post-surgery, proportionately, it is actually gained. While absolute lean body mass is lost, relative lean body mass is increased. Several

studies have found a strong correlation between plasma homocysteine and LBM.[18, 51, 153] Dierkes et al explained this finding by suggesting that since creatine is directly related to muscle mass, differences in creatine formation might be responsible for the differences in the formation of homocysteine.[51] Creatine is formed endogenously from methionine. Creatinine is the metabolic by-product of creatine, and creatinine is excreted in proportion to the amount of lean body mass. Therefore, according to Battezzati, a higher relative protein or lean body mass would lead to higher levels of methionine and homocysteine.[18] If this is true, it might offer some explanation as to why body mass loss was correlated with homocysteine gains in the current study, especially during times of greatest mass loss.

The findings that serum vitamin B-12 and folic acid levels had stronger correlation with homocysteine at baseline and twelve months might be related to the significant correlations between changes in serum homocysteine and loss in body mass over the first follow-up periods. It seems plausible that during times of the greatest mass loss the "normal" metabolism of homocysteine might be disturbed. However, this is purely speculative as enzyme activity and concentrations of intermediates in the homocysteine pathway, such as methionine synthase (MS), cystathionine synthase (CBS), methionine adenosyltansferase (MAT), and S-Adenosylhomocysteine hydrolase were not obtained.

This study has several limitations, including sample size and homogeneity of the population. There were only 19 women, with the majority being Caucasian, that were part of these analyses. Another limitation was that only one blood sample was analyzed per participant, at each time point. It has been shown that a single homocysteine sample

will only be within ±16.1% to ±18.4% of the individual's homeostatic set point with a 95% probability.[42, 69, 192] Measurement of multiple samples around a given time frame provide a smaller variation as there is day-to-day variability for an individual. If three to five homocysteine determinations were made around each time point, it has been shown that the mean will have a coefficient of variation of ±9% and ±7%.[42, 69, 192] It is important to note that biological variation is relevant in interpreting the data from the current study. Because there was only one measurement taken; there is greater chance that the homocysteine level recorded is not the participant's true serum level and more subject to day to day variability. Additionally, the study did not include a nonsurgical, stable, control group for comparisons. A control group would have provided information about homocysteine levels in the morbidly obese that do not have surgery.

Interestingly, there was one participant out of nineteen that experienced a significant drop in her serum homocysteine levels over the course of the study. Ths participant also had the highest baseline level of homocysteine (22.8 μM/L). This particular participant was the only subject that had a BMI of less than 40 kg/m^2 at baseline. Over twelve months, she lost close to 80% of her excess weight and finished the study with a BMI of 25.0 kg/m^2. This subject's vitamin status improved with each visit and could have also contributed to the decrease in serum homocysteine levels. While this is purely speculative, gastric bypass might have a significant lowering action on homocysteine levels in patients with hyperhomocysteinemic levels at baseline. It also could be possible that a reduction from an obese state to a 'normal' mass level will provoke a decrease in homocysteine. While this was only one patient, no conclusions can be made about her individual response. A larger sample size with more

hyperhomocysteinemic patients at baseline may have changed the results and might be a future research interest.

While the surgery did not decrease serum homocysteine levels as we hypothesized, homocysteine levels did not increase as they did in other studies in which vitamin status went uncontrolled. The maintenance of homocysteine levels after surgery in this study may be linked with maintaining adequate levels of vitamin B-12 and folic acid.

As the incidence of morbid obesity continues to rise, gastric bypass surgery will remain a viable treatment option for these individuals. Having a greater understanding of metabolic changes that occur, especially as they relate to comorbidities of obesity, including cardiovascular disease, is critical. Future research is needed to further understand the alterations in the metabolism of homocysteine during periods of rapid mass loss.

In conclusion, serum homocysteine levels did not significantly change during the study. 95% of the participants had normal homocysteine levels at baseline and throughout the course of the study and did not vary significantly. Change in homocysteine was significantly negatively correlated to change in BMI as well as change in weight between baseline values and practically all other visits. Results indicate that when vitamin status is controlled, Roux-en-Y gastric bypass surgery will not significantly alter homocysteine levels in morbidly obese patients.

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