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# Assessment of urinary methylmalonic acid levels in older adults on proton pump inhibitors

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ABSTRACT

ASSESSMENT OF URINARY METHYLMALONIC ACID LEVELS IN OLDER ADULTS ON PROTON PUMP INHIBITORS

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**Background:** Proton pump inhibitors (PPIs) affect gastric acid secretion. The use of PPIs in the elderly population (>70 years old) may increase the risk of vitamin $B_{12}$ deficiency because gastric acid is needed for vitamin $B_{12}$ digestion and absorption. However, it's unclear whether adults ages 50-70 years old who use PPIs chronically are at risk of vitamin $B_{12}$ deficiency.

**Objective:** To determine whether chronic use of PPIs results in increased urinary methylmalonic acid (uMMA) levels in adults ages 50-70 years old, indicating vitamin $B_{12}$ deficiency.

**Methods:** Fifteen men and women who had been taking PPIs daily for a minimum of one year were recruited. Fifteen subjects, not taking PPIs, were age-matched (± 3 years) and gender-matched to the subjects taking PPIs. Tissue stores of vitamin $B_{12}$ were determined using uMMA.

**Results:** There were no significant differences in uMMA levels between those taking PPIs (Mdn = 1.60 µg uMMA/mg creatinine) and those not taking PPIs (Mdn = 1.80 µg uMMA/mg creatinine) ($p = 0.12$).

**Conclusion:** Chronic use of PPIs did not alter vitamin $B_{12}$ status of subjects in healthy adults ages 50-70 years old. Regular monitoring of vitamin $B_{12}$ status does not appear to be needed in this age group; however studies using larger groups are indicated to confirm these findings.

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# ASSESSMENT OF URINARY METHYMALONIC ACID LEVELS IN OLDER ADULTS ON PROTON PUMP INHIBITORS

BY

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# CHAPTER 1

# INTRODUCTION

## Background

In the United States (U.S.), adults over 65 years of age comprise 13.7% of the population.[1] This is the equivalent of 43.1 million people.[1] This is a 21% increase since 2002 and more than triple the older adult population since 1900.[1] While this population has increased, another population group is steadily growing, and between 2002 and 2012, Americans ages 45-64 years old have increased by 24%.[1] The aging population often suffers from at least one chronic condition or possibly multiple co-morbidities including gastrointestinal issues. One common condition, affecting between 6-17% of the U.S. elderly population, is gastroesophageal reflux disease (GERD).[2] A common medication for GERD is a proton pump inhibitor (PPI). PPIs act by binding the hydrogen potassium ATPase pump of the parietal cells, thereby inhibiting gastric acid secretion from these cells in the stomach.[3] This lack of gastric acid is thought to have adverse effects on the absorption of vitamin $B_{12}$ bound to food sources since stomach acid is required to break the vitamin $B_{12}$/protein bonds. These bonds must be broken in order for vitamin $B_{12}$ to become available for absorption. The elderly are prone to additional gastric problems that may also decrease their ability to absorb nutrients.[4]

As much as 11-50% of the elderly population is thought to have chronic atrophic gastritis.[5] Atrophic gastritis results in malabsorption of vitamin $B_{12}$ as well as other nutrients.[5]

Since vitamin $B_{12}$ is vital for neurological functions, a deficiency may contribute to cognitive decline that is often seen in the elderly population. Vitamin $B_{12}$ deficiency is a common problem in the elderly. According to the data from the National Health and Nutrition Examination Surveys from 1999 to 2002, serum vitamin $B_{12}$ deficiency was seen in approximately 4% of people aged 40-59 years old and 6% of people > 70 years old.[6,7] Marginal status was even more common and occurred in approximately 14-16% of individuals ages 20-59 years old and >20% of individuals greater than 60 years old.[6] Deficiency is very unlikely to be linked to lack of dietary intake even within this vulnerable population and malabsorption is considered the most common culprit of vitamin $B_{12}$ depletion.[8] Currently, there are no standards for screening at-risk individuals for vitamin $B_{12}$ deficiency although some sources recommend a vitamin $B_{12}$ supplement >50 µg/day to prevent a deficiency in the elderly.[8,9] However, 50 µg/day would not be sufficient to replete levels for a person already suffering from a moderate vitamin $B_{12}$ deficiency.[8] Even if widespread screening was implemented, identifying vitamin $B_{12}$ deficiency in this population would be difficult, costly, and the current biomarkers that are utilized may not be the most sensitive or precise indicators.

Most studies have evaluated serum vitamin $B_{12}$ or serum methylmalonic acid (sMMA) to determine vitamin $B_{12}$ status, yet there are limitations to both of these biomarkers.[10,11] Research indicates that serum vitamin $B_{12}$ and sMMA may be flawed in identifying vitamin $B_{12}$ status because serum levels of vitamin $B_{12}$ and MMA may remain stable while tissue stores of vitamin $B_{12}$ are deficient, whereas urinary methylmalonic acid (uMMA) can assess acute tissue stores of vitamin $B_{12}$.[10] uMMA can be easily measured in the urine and is one of the earliest and most precise markers indicative of vitamin $B_{12}$ tissue depletion.[8,11] Only one study has utilized uMMA to determine vitamin $B_{12}$ status in younger individuals (20-50 years old) and did not find a

difference in uMMA between PPI users and non-PPI users.[12] Typical biomarkers used to evaluate vitamin $B_{12}$ status may not be sensitive enough to identify an early deficiency and thus prevent the occurrence of irreversible neurological symptoms.[13] uMMA has an overall greater sensitivity and specificity than other biomarkers, including sMMA, and should be considered a functionally relevant biomarker of acute tissue stores of vitamin $B_{12}$.[11,14]

The current body of research regarding vitamin $B_{12}$ absorption and PPI use in the elderly has shown conflicting results. A study analyzing uMMA in the younger population using PPIs did not find any significant differences in uMMA values.[12] Some studies in the elderly using serum vitamin $B_{12}$ as the biomarker have shown no effect of PPIs on vitamin $B_{12}$ status.[15,16] In contrast, other studies analyzing PPI use in the elderly show a decreased level of serum vitamin $B_{12}$ compared to non-PPI users.[17-19] The current research has also primarily focused on individuals >65 years old or <50 years old, ignoring the aging population between 50-65 years old. A majority of the current research used serum vitamin $B_{12}$ to detect a vitamin $B_{12}$ deficiency, and serum vitamin $B_{12}$ may miss a significant proportion of the population that may be deficient or have suboptimal vitamin $B_{12}$ status. No studies have analyzed uMMA in the aging or elderly population, likely due to the high cost of testing for uMMA. This research will investigate whether PPI use results in vitamin $B_{12}$ deficiency when using the biomarker uMMA in the 50-70-year-old adult population.

## Justification

The older adult population will continue to grow due to increased life expectancy, and by 2030 roughly 20% of the U.S. population will be comprised of older adults > 65 years old.[1] Adults over the age of 50 years old are more prone to developing GERD and as such are

commonly prescribed PPIs for long-term use. The adverse effects of PPIs have been reported, but studies are often inconclusive and long-term effects of PPI treatment remain largely unknown.[3,15,17,18,20-22] Vitamin $B_{12}$ deficiency can be a very serious condition leading to irreversible neurological damage, cognitive impairment, and dementia.[13] In addition, vitamin $B_{12}$ malabsorption may be further exacerbated by age-related gastric changes (i.e. hypochlorhydria) in the aging adult population.[4,8] Cognitive function in the elderly, as well as the aging population, is important and it may be markedly improved by providing a vitamin $B_{12}$ supplement, should a deficiency be detected.

## Statement of the Problem

The purpose of this investigation is to determine if aging adult PPI users have a greater occurrence of deficiency of vitamin $B_{12}$, as determined through uMMA, versus non-PPI users. The independent variable is the utilization of PPIs in older adults ages 50-70 years old. This includes both PPI users and non-PPI users. The dependent variable is the uMMA levels of both the PPI users and non-PPI users.

## Hypothesis

It is hypothesized that adult PPI users will have higher uMMA levels, indicating a vitamin $B_{12}$ deficiency, versus non-PPI users.

# CHAPTER 2

# REVIEW OF LITERATURE

## Vitamin $B_{12}$

### Dietary and Supplemental Sources

Vitamin $B_{12}$, also known as cobalamin, is a water-soluble vitamin endogenously synthesized by intestinal bacteria. However, endogenous synthesis alone in humans is not sufficient, so exogenous vitamin $B_{12}$ intake is essential to meet required needs.[8] Rich sources of vitamin $B_{12}$ include organ meats (i.e. liver and kidney), poultry, fish, shellfish, mushrooms, eggs, milk and dairy products.[8,23] Grain and cereal products, despite being of plant origin, are good sources of vitamin $B_{12}$ due to fortification practices in the U.S.[23] The fortification of various food items allows one to meet the daily Recommended Dietary Allowance (RDA). The RDA for vitamin $B_{12}$ is 2.4 µg/day, but 4-7 µg/day may be recommended based on existing vitamin body stores and other co-morbidities.[23]

Individuals may also be instructed to consume varying forms of crystalline vitamin $B_{12}$. Consuming this form of vitamin $B_{12}$ increases the bioavailability because it is not food-bound and therefore does not require gastric acid for digestion and absorption. For example, individuals ages 51 years and older are encouraged to consume fortified foods or supplements of vitamin $B_{12}$ because age-related gastric changes may impair the absorption of food-bound vitamin $B_{12}$ in

older adults.[8,23] Dietary intake of vitamin $B_{12}$ is usually adequate or in excess of needs in the U.S., especially for those consuming animal sources such as meat. Since intake is usually adequate, deficiency is much more likely to occur due to malabsorption.[4]

## Functions and Biochemistry

Enzymatic reactions rely on adequate exogenous intake of vitamin $B_{12}$ in humans. There are two active coenzyme forms of vitamin $B_{12}$, methylcobalamin and adenosylcobalamin.[8] The conversion of homocysteine to methionine via re-methylation and catalysis by methionine synthase requires methylcobalamin.[8,23] Initially, vitamin $B_{12}$ bound to methionine synthase acquires a methyl group from 5-methyl tetrahydrofolate (THF), whereas the 5-methyl-THF is the methyl group donor and vitamin $B_{12}$ acts as an intermediate acceptor of the methyl group.[8,23] The next step involves methionine synthase releasing the methyl group from the bound methylcobalamin to be transferred to homocysteine, resulting in the products methionine and vitamin $B_{12}$.[23] A deficiency of vitamin $B_{12}$ halts methionine synthesis and homocysteine accumulates, which may have adverse effects on cardiovascular health.[8] This two-step reaction makes other folate reactions occur due to the production of THF.[8] In the presence of a vitamin $B_{12}$ deficiency, the methyl form of THF accumulates and becomes inaccessible via the methyl-folate trap.[8,23] The methionine produced is also further activated to S-adenosylmethionine (SAM), and with a vitamin $B_{12}$ deficiency, there is a decreased conversion of methionine to SAM.[23] SAM is essential for methylation reactions, which are required for myelin maintenance. Myelin is an insulating layer, or sheath, that forms around the nerves and is responsible for transmitting electrical impulses in nerve cells.[24] If myelin is not maintained, neural function may become compromised, resulting in neuropathy or other neurodegenerative disorders.[13,23] This

explains the neurological symptoms that accompany a vitamin $B_{12}$ deficiency. Figure 1 depicts the re-methylation of homocysteine to form methionine and the role of vitamin $B_{12}$ in this reaction.[23]

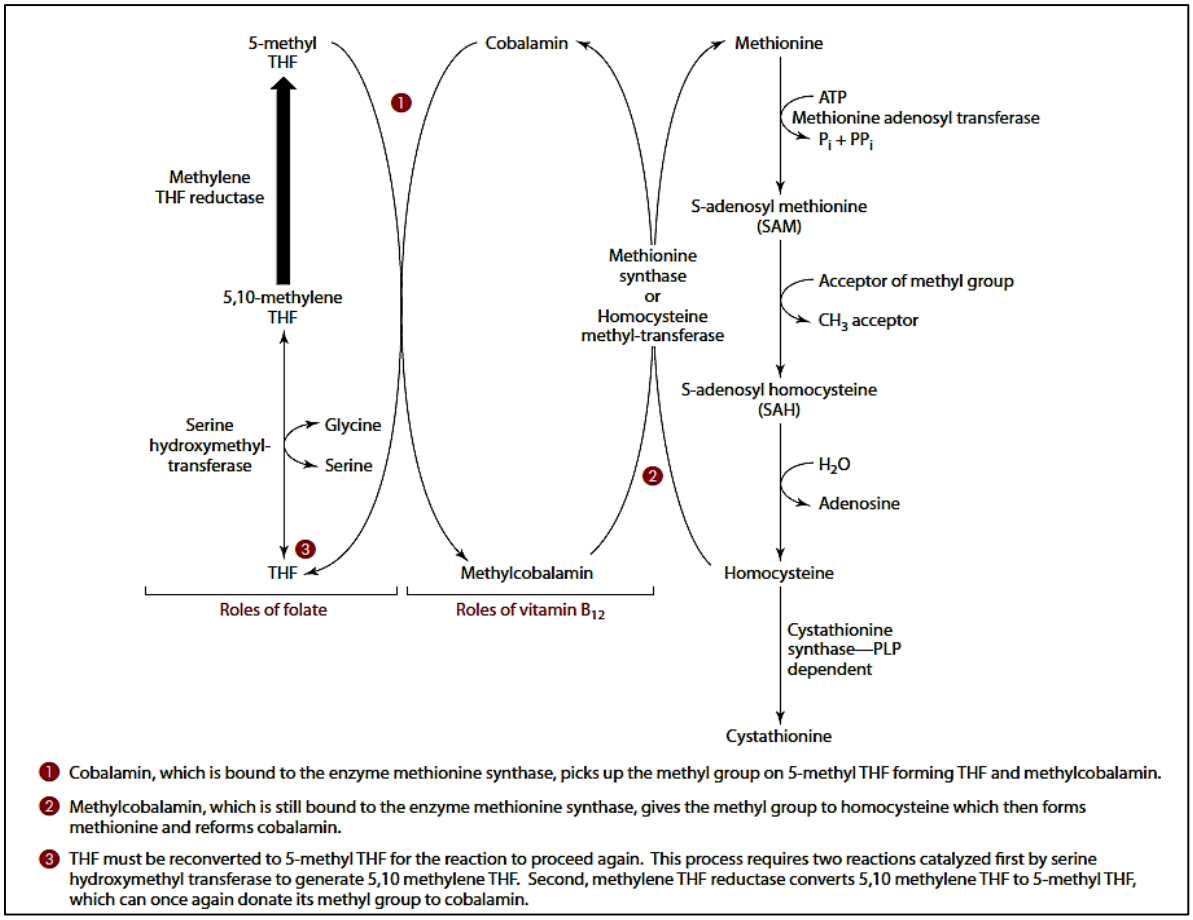


Figure 1: Conversion of homocysteine to methionine

The enzyme methylmalonyl-CoA mutase requires the coenzyme adenosylcobalamin in the form of 5-deoxyadenosylcobalamin for the reaction converting L-methylmalonyl-CoA to succinyl-CoA.[9,23] This allows succinyl-CoA to be formed and proceed to enter the citric acid cycle, which helps to produce energy from proteins and lipids.[9,23] Succinyl CoA is also required

for the formation of hemoglobin in the red blood cells.[9,23] Two units of adenosylcobalamin are required for this reaction, and with a vitamin $B_{12}$ deficiency, the mutase activity will be impaired and MMA will accumulate in the body fluids such as the serum and urine. uMMA can be used to evaluate vitamin $B_{12}$ tissue depletion and cellular vitamin $B_{12}$ depletion.[8,11,23] Figure 2 shows the formation of succinyl-CoA and the role of vitamin $B_{12}$ in this reaction.[23]

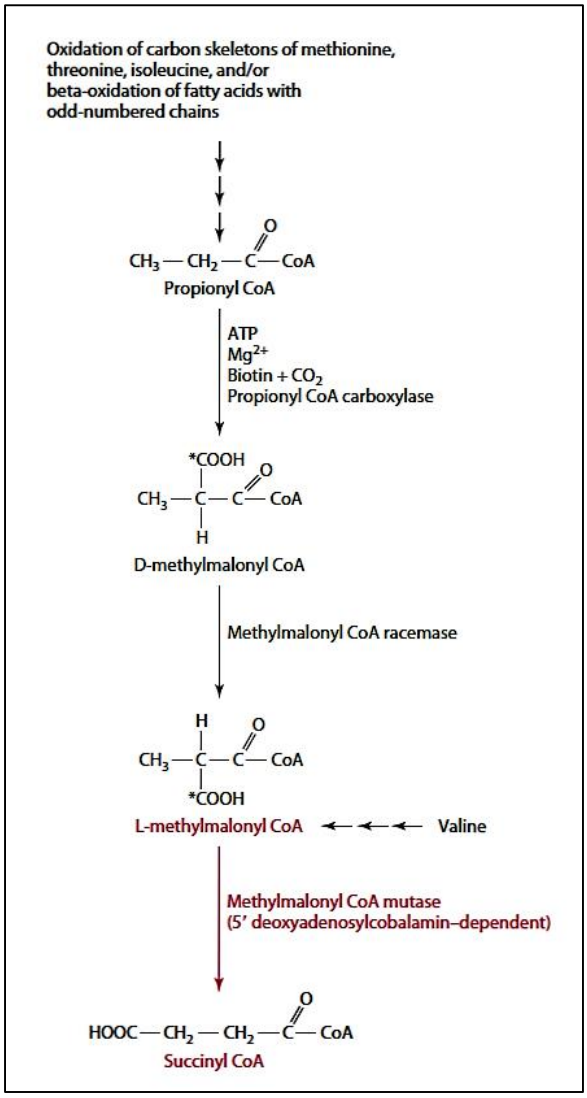


Figure 2: Formation of succinyl-CoA

## Absorption, Metabolism and Storage

Vitamin $B_{12}$ absorption begins in the stomach where food-bound vitamin $B_{12}$ is released from proteins by gastric hydrochloric acid ($HCl^-$) and the gastric proteolytic enzyme, pepsin.[8,13,23,25] After the release of vitamin $B_{12}$, the free vitamin $B_{12}$ is bound to an R protein (haptocorrin or transcobalamin I) from saliva and is then transported to the duodenum.[8,23] This R protein is thought to provide protection to vitamin $B_{12}$ from use by intestinal bacteria.[23]

Next, the vitamin $B_{12}$/R protein complex is acted upon by pancreatic proteases, thereby releasing vitamin $B_{12}$ from the R protein. Intrinsic factor (IF), a glycoprotein produced by gastric parietal cells, will only bind to free vitamin $B_{12}$ in a neutral environment made so by the pancreatic proteases.[23,25] IF is specific for vitamin $B_{12}$ and also protects vitamin $B_{12}$ from use by intestinal bacteria.[8] The vitamin $B_{12}$/IF is carried from the proximal small intestine to the terminal ileum where it is taken up by the protein receptor, cubilin.[8,13,23] The proteins amnionless and megalin associate with the vitamin $B_{12}$/IF complex and facilitate the attachment of cubilin to the ileal cell plasma membrane.[8,13,23] This interaction of the vitamin $B_{12}$/IF complex and cubilin initiates a $Ca^{2+}$-dependent receptor-mediated endocytosis which leads to internalization of the complex.[8,13,23] Within the enterocyte, vitamin $B_{12}$ is released from IF due to the low pH within the endosomes and lysosomes and then proceeds to bind with transcobalamin II for transport into the portal blood.[8,23] Absorption of vitamin $B_{12}$ occurs primarily in the distal ileum, and while passive diffusion may occur at very high concentrations, it is an inefficient process.[13,23]

Vitamin $B_{12}$ is stored primarily in the liver as adenosylcobalamin in the amount of approximately 2-4 mg.[23,25] Vitamin $B_{12}$ is also enterohepatically recycled, thus making it

available for binding with IF and absorption in order to maintain the body's need for vitamin $B_{12}$.[25] This enterohepatic circulation contributes to the long biological half-life of vitamin $B_{12}$. A deficiency of vitamin $B_{12}$ due to insufficient intake would take a long length of time to develop, approximately 3-5 years.[23,25] The length of time for the development of vitamin $B_{12}$ deficiency could change if there is an underlying condition or disease state such as lack of gastric acid or pernicious anemia, in which IF is lacking.[25]

## At-Risk Populations for Deficiency

Vegetarians and vegans are at the greatest risk of vitamin $B_{12}$ deficiency due to the lack of animal product consumption. Individuals with gastrointestinal diseases such as Zollinger-Ellison syndrome or Crohn's disease are also at a higher risk of deficiency due to malabsorption.[13] Certain medications may increase the risk of developing vitamin $B_{12}$ deficiency, such as PPIs and metformin, which is used to help control blood sugar in people with Type II diabetes.[13] Older adults comprise a significant proportion of the U.S. population and are at an especially increased risk of developing a vitamin $B_{12}$ deficiency. The older adult population experience a greater risk due to gastric changes associated with aging, such as chronic atrophic gastritis.[8] Older adults are commonly prescribed medications such as PPIs and metformin, thereby making the risk of vitamin $B_{12}$ malabsorption even greater. In studies regarding older patients with vitamin $B_{12}$ deficiency, Grober et al. state that 53% of cases were due to malabsorption while 33% were due to anemia and only 2% due to a lack of dietary intake.[13] A Dutch study examined 105 healthy, free-living elderly persons and concluded that ≥23.8% of the population had a mild vitamin $B_{12}$ deficiency.[26] Vitamin $B_{12}$ deficiency was explained by low

exogenous intake or severe atrophic gastritis in only 28% of the deficient population, leaving the remaining cases of vitamin $B_{12}$ deficiency unexplained.[26]

## Causes of Deficiency

Malabsorption is considered to be the most common cause of vitamin $B_{12}$ deficiency, especially in the elderly.[4,8] Malabsorption may be caused by many reasons, including chronic atrophic gastritis, gastrointestinal diseases or certain drugs and medications.[4,8,13,23] Two types of chronic atrophic gastritis exist, Type A and Type B, with Type B being the most common.[8] Type A atrophic gastritis ultimately results in a lack of IF due to an autoimmune response that destroys parietal cells and develops into megaloblastic or pernicious anemia.[5,8,27] Type A chronic atrophic gastritis is uncommon in the general population and predominates in the elderly population; it is still relatively rare in the elderly.[27] The prevalence of Type A chronic atrophic gastritis is approximately 5% of the U.S. population.[5] This condition is characterized by atrophy of the body of the stomach while sparing the antrum.[8,27] This results in achlorhydria or hypochlorhydria and the destruction of parietal cells due to an autoimmune response producing antibodies to gastric parietal cells leading to a loss of IF.[8,27] Type A atrophic gastritis ultimately may develop into pernicious anemia, which results from the lack of IF.

Type B atrophic gastritis is characterized by a decrease in gastric acid secretion, and studies suggest 20-50% of the elderly are subject to this type of gastritis.[8] Type B chronic atrophic gastritis is characterized by reduced gastric acid secretions and reduced pepsinogen, and IF may become reduced in very severe, advanced stages.[5,8] Type B gastritis is thought to develop as an end result due to a chronic infection of *H. pylori*.[5] *H. pylori* decreases the gastric acidity in

the antrum of the stomach and this decrease in acidity allows the infection to spread to the body of the stomach, increasing bacterial colonization and hastening the progression of gastritis.[4,5]

Gastrointestinal diseases such as Zollinger-Ellison's syndrome, Crohn's disease, and ulcerative colitis may result in malabsorption of nutrients including vitamin $B_{12}$.[13] Zollinger-Ellison's syndrome is characterized by an increased release of gastric acid resulting in a more acidic environment in the small intestine and therefore not allowing vitamin $B_{12}$ to unbind from the R protein and onto IF and ultimately causing the vitamin $B_{12}$ to be unavailable for absorption.[23]

Bacterial overgrowth caused by an increased pH of the stomach and further alkalization of the small intestine due to achlorhydria may also contribute to vitamin $B_{12}$ deficiency because competition for the uptake of vitamin $B_{12}$ may occur.[8,13] Common bacterial overgrowth often includes *Campylobacter, Yersinia*, and *Clostridium.*[8] *H. pylori* infection may also have a negative effect on vitamin $B_{12}$ absorption by contributing to the development of chronic atrophic gastritis.[8,25] The destruction of parietal cells via the *H. pylori* infection and therefore the reduction of gastric acid secretions may increase the pH of the stomach and decrease the bioavailability of protein bound vitamin $B_{12}$.[25] This is especially seen in the elderly with approximately 60% of the elderly > 60 years old being infected with *H. pylori.*[8] One study observed a normalization of vitamin $B_{12}$ levels after treatment with antibiotic therapy, suggesting that bacterial overgrowth may have contributed to the vitamin $B_{12}$ deficiency.[5]

Certain medications may contribute to a vitamin $B_{12}$ deficiency by promoting gastric changes.[8] PPIs inhibit gastric acid secretion and may contribute to food-bound vitamin $B_{12}$ malabsorption.[8] PPIs will be discussed in greater depth in the next section. Histamine$_2$ receptor antagonists ($H_2$RA) such as cimetidine, famotidine, and ranitidine are used to treat peptic ulcer

disease and may affect the absorption of vitamin $B_{12}$; however, it is unclear whether these drugs may cause an overt deficiency.[9] The drug metformin may also contribute to vitamin $B_{12}$ deficiency. This drug may inhibit vitamin $B_{12}$ absorption by binding free calcium required for absorption of the vitamin $B_{12}$/IF complex in the terminal ileum, although research is still needed to determine the severity of this effect.[9] Other drugs known to affect vitamin $B_{12}$ status include cholestyramine, a bile acid-binding resin used to treat high cholesterol, and the antibiotics chloramphenicol and neomycin.[8] An anesthetic, nitrous oxide, may produce clinical vitamin $B_{12}$ deficiency symptoms because it oxidizes and disables vitamin $B_{12}$ and therefore its related enzyme activity.[9,13,23] The use of nitrous oxide should be used cautiously in individuals with a higher risk of pre-existing vitamin $B_{12}$ deficiency.[9] It is also important to be observant of folic acid supplementation because this may mask a vitamin $B_{12}$ deficiency and lead to possible irreversible neurological damages.[9]

Although malabsorption is the primary cause of vitamin $B_{12}$ deficiency, inadequate dietary intake may also lead to a vitamin $B_{12}$ deficiency. Dietary intake of vitamin $B_{12}$ has been shown to exceed the RDA in the U.S., but people following a vegetarian or vegan diet may be at the highest risk for deficiency. Vitamin $B_{12}$ is mostly found in animal products and therefore may contribute to a vitamin $B_{12}$ deficiency if these foods are avoided and a supplement is not untilized.[8] Insufficient dietary intake may be a concern in the elderly population, but it is not the usual cause of deficiency. Wolters et al. found the mean intake of vitamin $B_{12}$ in 174 women 60-70 years old to be 5.1 µg/day, clearly exceeding the RDA.[8] Even in the presence of inadequate intake, a deficiency may take 3-5 years to develop because vitamin $B_{12}$ is stored long term in the liver and is secreted in bile for reabsorption to attenuate for the lack of intake.[8,23]

## Signs and Symptoms of Deficiency

Overt vitamin $B_{12}$ deficiency is less common and almost all cases involve mild biochemical changes without the classic clinical manifestations such as anemia.[28] Vitamin $B_{12}$ deficiency progresses in four main stages. The first stage is characterized by low serum levels of vitamin $B_{12}$ on transcobalamin II, although it is important to note that serum levels may remain relatively normal until storage is completely exhausted.[8,23] The next stage involves the depletion of cellular vitamin $B_{12}$ shown by low levels of transcobalamin II, holohaptocorrin, erythrocyte vitamin $B_{12}$ concentrations, and dysfunction of vitamin $B_{12}$-dependent enzymes.[8,23] The third stage results in a biochemical deficiency with a decrease in deoxyribonucleic acid (DNA) synthesis and an increase in plasma homocysteine and MMA.[8,23] The final stage manifests as a clinical deficiency wherein blood cells are changed functionally and morphologically, resulting in megaloblastic macrocytic anemia and neuropsychiatric disorders.[8,23]

The consequences of vitamin $B_{12}$ deficiency are both hematological and neurological. The Institute of Medicine (IOM) reports that 75-90% of people with clinical vitamin $B_{12}$ deficiency present with neurological disorders, and this is the only expression of deficiency in 25% of cases.[13] The hematological symptoms include anemia, low blood leukocyte and thrombocyte counts, paleness of the skin, fatigue, shortness of breath and palpitations.[23] Megaloblastic macrocytic anemia occurs in the final stage of deficiency due to the methyl folate trap that occurs as a consequence of vitamin $B_{12}$ deficiency.[23] Without vitamin $B_{12}$, the folate coenzymes are reduced to the 5-methyl THF form and cannot be converted to the active coenzyme form for DNA synthesis.[23] This negatively affects rapidly producing cells such as the red blood cells, thus resulting in anemia.[23]

The neurological symptoms of deficiency may be irreversible if not addressed in a timely manner.[13,23] A vitamin $B_{12}$ deficiency causes demyelination resulting in neurological symptoms that include numbness in the extremities, unsteady gait, and lack of coordination and spacial perception.[13,23] Other symptoms of cerebral disorders also may be seen including confusion, apathy, memory loss, depression, dementia, psychosis, disorientation and irritability.[13,23] The neurological symptoms are seen in approximately 75-90% of deficiency cases and are not responsive to treatment with folate supplementation.[23] Interestingly, there appears to be an inverse relationship between severity of hematological and neurological symptoms with vitamin $B_{12}$ deficiency.[13] Grober et al. argue that the neurological symptoms of deficiency are currently largely ignored and unrecognized in clinical practice.[13]

As previously mentioned, homocysteine levels will rise in the presence of a vitamin $B_{12}$ deficiency and this may result in cardiovascular complications such as atherosclerotic disease, coronary artery disease, stroke, and thrombosis.[5,29] The Framingham Heart Study cohort concluded that serum vitamin $B_{12}$ levels below 296 pmol/L are associated with an increase in homocysteine levels.[5] The Rotterdam Scan Study evaluated a 60-90 years old population and found that total homocysteine levels were directly associated with silent brain infarcts and white-matter lesions.[29] Seshadri et al. also evaluated the Framingham Heart Study cohort and determined that increased plasma homocysteine seemed to be a strong independent risk factor for the development of dementia.[30] The relative risk of developing dementia was 1.4 for each increase in 1 standard deviation above the mean and the relative risk of Alzheimer's disease was 1.8.[30] Plasma homocysteine levels above 12 µmol/L nearly doubled the risk of Alzheimer's disease.[30] Clinical trial data have shown that brain atrophy and cognitive decline slows with supplementation of folic acid, vitamin $B_{12}$, and vitamin $B_6$ and this may be related to the B-

vitamin's previously mentioned ability to allow for re-methylation or transformation of homocysteine.[28] Increased levels of homocysteine seem to have not only detrimental effects regarding cardiovascular disease, but cognitive disorders as well.

## Biomarkers

There are several biomarkers that may be used to evaluate vitamin $B_{12}$ levels in the body. The most common biomarkers include serum vitamin $B_{12}$ levels, red blood cell mean corpuscular volume (MCV), holotranscobalamin (holoTC) and serum and urinary methylmalonic acid (sMMA, uMMA), respectively.

Serum vitamin $B_{12}$ levels are assessed in the laboratory utilizing either radioimmunoassay or chemiluminescense, meaning that normal ranges will be different depending on the assay method used.[10] Chatthanawaree states that serum vitamin $B_{12}$ may not be a good indicator of vitamin $B_{12}$ deficiency or tissue depletion due to its variability.[10] Generally speaking, borderline levels are 200-300 pg/mL and vitamin $B_{12}$ deficiency can be indicated by levels <200 pg/mL.[8,10,13] Wolters et al. state that this level may be maintained even once the tissue stores of vitamin $B_{12}$ are exhausted.[8] Thus, serum vitamin $B_{12}$ levels <300 pg/mL, and <350 pg/mL for the elderly, should be verified with other tests.[8] It is also important to note that a functional vitamin $B_{12}$ deficiency may be present with serum levels up to 450 ng/L.[13] The lack of serum sensitivity for vitamin $B_{12}$ suggests that metabolite testing be used in conjunction with serum measurements when diagnosing a vitamin $B_{12}$ deficiency.[10]

MCV can be utilized because a methylcobalamin deficiency causes a build-up of the inactive form of folate (5-methyltetrahydrofolate) and a decreased level of active folate (tetrahydrofolate) in the body.[10] This biomarker is not specific to vitamin $B_{12}$ or folate. Levels

greater than 130 fL are 100% indicative of a deficiency of either vitamin $B_{12}$ or folate, but an elevation in MCV is a relatively late sign of a deficiency and not a good indicator for a vitamin $B_{12}$ deficiency diagnosis.[8,10]

Holotranscobalamin is a biomarker that becomes reduced with a vitamin $B_{12}$ deficiency. This is the active part of the vitamin $B_{12}$ that delivers the vitamin to the body cells and is thought to be the earliest marker for a vitamin $B_{12}$ deficiency, but limitations include its short half-life in the plasma and poor specificity.[10,13] A level of <35-45 pmol/L is used to indicate deficiency of vitamin $B_{12}$.[10,13]

MMA is the hallmark of vitamin $B_{12}$ deficiency and a highly sensitive functional indicator that indicates tissue depletion.[8,10,11,13] If a vitamin $B_{12}$ deficiency is present, a lack of adenosylcobalamin will be present and this will cause an accumulation of D-methylmalonyl-CoA, which is then converted to MMA. MMA can be measured in both the serum and urine.[10] This test may be costly due to the equipment needed such as a mass spectrometer to analyze the serum or urine, but this metabolic byproduct can be used to detect tissue levels of vitamin $B_{12}$ before serum vitamin $B_{12}$ measures would indicate such a deficiency.[8,10,13] Normal levels for sMMA are 70-270 nmol/L.[10] Lukaszuk et al. used uMMA in their analysis of vitamin $B_{12}$ levels in non-elderly individuals and defined normal levels of uMMA <3.8 µg MMA/mg creatinine (<3.6 µmol/mol creatinine).[12,31]

One study screened for low uMMA at various health fairs, retirement communities and other locations geared towards the elderly population. uMMA was taken from 809 individuals and 36 participants had elevated uMMA, defined as > 5.0 µg MMA/mg creatinine.[32] Of these 36 participants, only 18 subjects had low serum vitamin $B_{12}$ while 17 subjects had low-normal or normal vitamin $B_{12}$ levels.[32] Twenty-three subjects then received intramuscular vitamin $B_{12}$ and

16 subjects were seen after the treatment. All these participants now had a statistically significant decrease in uMMA levels showing that uMMA responds to changes in vitamin $B_{12}$ levels and that this biomarker is more sensitive in detecting a vitamin $B_{12}$ deficiency.[32] Another study analyzing the dose response of vitamin $B_{12}$ supplementation of uMMA levels recruited individuals with poor vitamin status defined as plasma vitamin $B_{12}$ <250 pmol/L and uMMA ratio >1.5 µmol MMA/mmol creatinine.[11] Hill et al. observed that with supplementation of vitamin $B_{12}$, uMMA levels decrease rapidly within the first 2 weeks of supplementation with no further decrease thereafter, suggesting that correcting the metabolic effects of poor vitamin $B_{12}$ status can be done fairly quickly, especially with a high supplemental dose.[11]

Although the sMMA and uMMA respond similarly to supplementation, the uMMA is superior to that of sMMA due to its increased sensitivity for vitamin $B_{12}$ status.[11] sMMA may be falsely elevated due to renal insufficiency while uMMA does not show much variation; however, creatinine should be utilized when interpreting uMMA to account for possible kidney function impairment.[13,31] uMMA is also 40 times more concentrated than sMMA and the test is non-invasive and only requires 1 mL of urine, which may be frozen for months.[14,31] False positives have not been reported with uMMA.[14] Both the serum and urinary MMA biomarkers have a higher sensitivity and specificity than serum vitamin $B_{12}$.[10] A potential drawback is the need for implementation of a quality assurance system regarding the uMMA measurements. Researchers from the Hospital of the University of Munich, Germany, found a deviation of >20% from the mean concentration of uMMA samples in a large proportion of samples submitted by ten European clinical laboratories.[33] This may not have implications for research in the U.S., though, and one can easily control for this variable by having all samples evaluated by one lab.

## Prevalence of Deficiency in the Aging Population

Vitamin $B_{12}$ deficiency or suboptimal levels in the elderly are relatively common, but little research focuses on vitamin $B_{12}$ deficiency in the 50-70 years old population. Although vitamin $B_{12}$ deficiency is common in the aging population, deficiency is not seen as a direct consequence of aging. The mechanism producing vitamin $B_{12}$ deficiency in the elderly seems to be due to an increased susceptibility due to accompanying disease states or use of medications that contribute to malabsorption.[27]

Diagnosis of vitamin $B_{12}$ deficiency varies greatly due to differing criteria for defining deficiency, but deficiency is more prevalent with older age and among Caucasians and Latinos.[10] When evaluating studies from 2005-2007, Chatthanawaree states the reported prevalence varies from 0.6 to 46%.[10] Desirable status for the elderly using serum vitamin $B_{12}$ is 220-258 pmol/L (300-350 pg/mL), and when used in conjunction with more sensitive biomarkers such as homocysteine and MMA, the prevalence of vitamin $B_{12}$ deficiency in the elderly is roughly 43%.[8] Vitamin $B_{12}$ status was observed in the Framingham Heart Study cohort elderly population aged 67-96 years old using both serum vitamin $B_{12}$ and metabolic markers such as sMMA and homocysteine; 40.5% had serum vitamin $B_{12}$ concentrations <258 pmol/L, and sMMA and homocysteine concentrations were markedly elevated along with low vitamin $B_{12}$ levels in 11.3% of the elderly participants.[34] This indicates that greater than 12% of this particular ambulatory, elderly population had vitamin $B_{12}$ deficiency and many of these individuals had normal serum vitamin $B_{12}$ levels, indicating the need to test metabolic markers and not solely serum levels of vitamin $B_{12}$.[34] Various studies from around the world have found vitamin $B_{12}$ deficiency to occur in between 7% to 30% in the elderly population in various settings including

free-living, assisted-living and skilled care.[13,26,35-37] It is also important to note that intake may be adequate, but a deficiency may still exist due to the many functional factors that may inhibit absorption and contribute to the development of a vitamin $B_{12}$ deficiency.[13] The research shows that vitamin $B_{12}$ deficiency seems to increase with age, thus contributing to the importance of identifying a deficiency earlier in the 50-70 years old population to address any low levels prior to progression of deficiency.

## Proton Pump Inhibitors

PPIs are pharmacological agents with gastric-acid-suppressing characteristics. Examples of PPIs include omeprazole, lansoprazole, rabeprazole, pantroprazole and esomeprazole.[4] PPIs have shown to be far superior to $H_2$RA because they are more potent suppressors of gastric acid.[38] PPIs were introduced in the mid to late 1980's and are generally viewed as safe and effective.[3,25] However, consequences of long-term use are not completely understood and potential long-term consequences such as vitamin $B_{12}$ malabsorption are of great concern in vulnerable populations such as the elderly.[25]

### Indications and Uses

PPIs are clinically indicated for use in GERD, esophagitis, acid hypersecretory states such as Zollinger-Ellison syndrome, peptic ulcers, *H. pylori* infection, dyspepsia and prophylaxis of peptic ulcers.[22,25,38] These drugs are often recommended as a first line of therapy for more serious conditions such as erosive esophagitis and Barrett's esophagus.[4,38] PPI use is also indicated in individuals taking other medications such as non-steroidal anti-inflammatory drugs

(NSAIDs), low-dose aspirin (up to 325 mg/day), corticosteroids, antiplatelet drugs and anticoagulants, which may increase risk of upper GI bleeding.[4,22,25,38]

There is concern regarding the potential overutilization of PPI therapy. Inappropriate prescription of PPI is defined by lack of medical indication, inappropriate duration of treatment, and inappropriate dose.[4] Inappropriate PPI use is not uncommon and some studies report that inappropriate use is seen in approximately 50-80% of elderly patients admitted and discharged from geriatric wards.[4] Elderly patients often remain on PPIs after *H. pylori* infection is totally eradicated. It is reported that 50-60% of PPI users for treatment of *H. pylori* infection became chronic PPI users.[4] This indicates many individuals, especially the elderly, may needlessly become chronic PPI users, thereby increasing their risk of nutrient malabsorption.[39] PPIs are also available over-the-counter without a prescription and this allows individuals to self-prescribe, leading to improper use in many cases.[39]

## Mechanism of Action

PPIs suppress gastric acid output via selective inhibition of the hydrogen potassium ATPase pump.[40] PPIs require activation by parietal cells in the presence of gastric acid leading to formation of a sulfonamide moiety which then irreversibly binds to the alpha subunit of the hydrogen potassium ATPase pump on the secretory canaliculus of actively secreting parietal cells, thus inhibiting the ability of parietal cells to produce hydrochloric acid.[3,40] The reduced gastric acid output of the parietal cells leads to increased gastric pH that causes the inhibition of somatostatin release from D-cells in the gastric antrum.[3] The lack of somatostatin release allows gastrin to be released from G-cells completely unrestrained. This leads to increased serum levels

of gastrin.[3] PPIs are metabolized primarily by the cytochrome P450 enzymes in the liver and then excreted predominantly via the urine.[3]

## Potential Side Effects

Common side effects of PPI use include headache, diarrhea, constipation, nausea and rash, but these are rather infrequent and mild.[4] Other less overt side effects exist regarding absorption of nutrients with long-term use and may have a larger impact on overall health.

### Nutritional Deficiencies - Vitamin $B_{12}$

There are different mechanisms that may be working to affect vitamin $B_{12}$ absorption. A lack of gastric acid can inhibit the release of dietary vitamin $B_{12}$ from food because gastric acidity is required to activate pepsinogen to pepsin which is required to liberate protein-bound vitamin $B_{12}$ from food sources, thus decreasing the bioavailability of vitamin $B_{12}$.[4,8,13,22,25,41] Gastric acid itself also helps to free vitamin $B_{12}$ from food, and if this is suppressed, absorption may be affected. Furthermore, gastric and small intestinal bacterial overgrowth may occur due to the increased pH in the stomach and the small intestine and these displaced bacteria can compete with the gut for absorption and use of vitamin $B_{12}$. A recent review concluded that gastric bacterial overgrowth has not been shown to have nutritional consequences, although uncertainty regarding this conclusion still exits.[4,8] Another suggested mechanism is the destruction of parietal cells, which produce IF, leading to the development of pernicious anemia by the inability of IF to bind vitamin $B_{12}$ and become absorbed.[25] However, pernicious anemia is relatively uncommon and decreased IF has not been seen with PPI use.[25]

Rebound Acid Hypersecretion

Rebound acid hypersecretion refers to the increase in gastric acid secretion after PPI treatment has been discontinued.[22] PPI therapy causes a decrease in gastric acid secretion and release of somatostatin by antral D-cells which then continues to an increased amount of gastrin released from G-cells.[3,22] Hypergastrinemia ensues and this increases the functional capacity of enterochromaffin-like cells and parietal cells, thereby leading to increased gastric acid secretion.[22] Sustained hypergastrinemia can increase acid secretion, but it does not become evident until cessation of PPI therapy, when symptoms reoccur with increased severity. This increased severity of gastric acid secretion often leads to chronic PPI therapy.[22,41]

## Relationship Between PPI Use and Vitamin $B_{12}$

The current body of research regarding vitamin $B_{12}$ deficiency and use of acid-lowering medications such as PPIs and $H_2$RAs is conflicting and results are varied. Lukaszuk et al. compared vitamin $B_{12}$ status in non-elderly (22-50 years old) chronic PPI users (daily use of PPIs for at least 1 year) who were age- and gender-matched with non-PPI users serving as the control group.[12] No significant differences were found between uMMA in PPI users and their matched controls.[12] This study was unique because it was the first to examine uMMA levels in non-elderly individuals; however, this study was limited by a small sample size.[12] A 2010 study analyzed institutionalized individuals between 60-89 years old regarding vitamin $B_{12}$ status in PPI users (n=17) and non-PPI users (n=19). Groups were not age- and gender-matched as in the previous study, but PPI usage was characterized as continuous use of PPIs for at least 1 year.[17] At baseline, there was a significant difference in the frequency of vitamin $B_{12}$ deficiency in PPI

users and non-PPI users, with 11 of 16 deficient in the PPI group and 2 of 18 deficient in the non-PPI group.[17] The entire PPI group were then supplemented with a nasal vitamin $B_{12}$ spray of 500 µg once per week for eight weeks. Pre- and post-serum vitamin $B_{12}$ levels and sMMA were collected. At conclusion of the study, the vitamin $B_{12}$ nasal spray increased serum vitamin $B_{12}$ levels in the PPI users and decreased the number of vitamin $B_{12}$-deficient institutionalized individuals.[17] The frequency of deficiency from baseline to 8 weeks was significant with 10 of 13 PPI users being deficient at baseline.[17] Only five PPI users remained deficient after 8 weeks of treatment with vitamin $B_{12}$ nasal spray.[17] This study was also limited by a small sample size, similar to Luksaszuk et al., and neither a food record nor food frequency was obtained in the study. There was also no placebo control group regarding the supplementation with nasal vitamin $B_{12}$ spray because it is not ethical to withhold treatment from individuals with a known vitamin $B_{12}$ deficiency.[17]

Three studies were published in 2008 and their conclusions varied. A cross-sectional study by Den Elzen et al. aimed to investigate the suspected association between long-term PPI use and abnormal vitamin $B_{12}$ status in elderly individuals.[16] Long-term PPI use was characterized by >270 defined daily doses per year for 3 years and elderly was characterized by ages ≥65 years old. The PPI user must have had a partner such as a husband, wife or child that met the inclusion criteria and had not used a PPI within the last 3 years or received parenteral vitamin $B_{12}$ treatment. These partners were not age- and gender-matched as with the previous studies mentioned. The purpose of these pairs was to control for dietary intake of vitamin $B_{12}$. One hundred twenty-five couples were identified that qualified to participate in this study. Blood sampling was performed to test serum levels of vitamin $B_{12}$, serum levels of homocysteine and MCV. Overall, no association was found between long-term PPI use and poor vitamin $B_{12}$ status

in elderly individuals in this study.[16] This study was not limited by a small sample size or short duration of PPI use, as users had been on PPIs for at least 3 years, but the authors suggest that a "healthy user" effect may have been at play due to the exclusion criteria.[16] Another issue could have been the lack of matching for age and gender and the biomarkers used to measure vitamin $B_{12}$ status because serum vitamin $B_{12}$, homocysteine and MCV are not sensitive or specific indicators of vitamin $B_{12}$ status. This study was also conducted in a community-living older adult population which may differ from institutionalized older adults.

Two other 2008 studies indicated that prolonged PPI use did indeed negatively affect serum vitamin $B_{12}$ levels. The cross-sectional study by Dharmarajan et al. aimed to examine the relationship between serum vitamin $B_{12}$ levels and use of PPIs and $H_2$RA in older adults ages 60-102 years old over a duration of 6 years.[18] The sample of participants was taken from a long-term care facility and a community health center in the Bronx, New York. Five hundred forty-two patients were eligible to be included in the data and information was collected about demographics, PPI or $H_2$RA use, and vitamin and mineral supplement use.[18] The results indicated that low or marginal vitamin $B_{12}$ status was observed in older adults with 19.9% in the nursing-home population and 29.2% in the community-living population.[18] The results showed that PPI use up to 72 months was associated with lower serum vitamin $B_{12}$ levels for both supplement and non-supplement users.[18] This decrease in serum vitamin $B_{12}$ levels was not seen with the use of $H_2$RA.[18] Vitamin $B_{12}$ supplements of 7-50 µg/day did not prevent lower vitamin $B_{12}$ levels, but it did delay the occurrence of vitamin $B_{12}$ deficiency.[18] Food records to analyze dietary intake were not collected, which may have been a limitation. The authors concluded that deficiency is indeed common among the older adult population, but special attention may be necessary to monitor vitamin $B_{12}$ levels in older adults utilizing PPIs.[18]

A study by Hirschowitz et al. followed up a prior study following acid hypersecretors with or without Zollinger-Ellison syndrome. In the prior study, subjects were treated with lansoprazole and results showed this PPI effectively decreased gastric acid secretion.[15] The follow-up study aimed to determine prevalence of vitamin $B_{12}$ deficiency in very long-term PPI users measuring serum vitamin $B_{12}$, sMMA, and homocysteine. This is significant because prior to this study, the sMMA and homocysteine tests had not been utilized in previous studies of vitamin $B_{12}$ malabsorption in omeprazole-treated patients. One finding was that the patients with altered homocysteine or sMMA levels showed a normalization of these levels when supplemented with vitamin $B_{12}$.[15] Utilizing these additional tests (MMA and homocysteine), subclinical vitamin $B_{12}$ deficiency was discovered in 31% of patients with normal serum vitamin $B_{12}$ levels.[15] Hirschowitz et al. did not find that prolonged or excessive acid or pepsin suppression was the explanation for the vitamin $B_{12}$ deficiency observed, indicating that another mechanism may be responsible, which continues to remain unknown. Hirschowitz et al. did not focus on elderly individuals, but subjects were utilizing PPIs long term and the average age was between 52.9-56.3 years.[15]

A 2004 case-control study investigated the association between vitamin $B_{12}$ deficiency and use of $H_2$RA and PPIs in older adults ≥ 65 years old.[19] Inclusion criteria included a documented serum vitamin $B_{12}$ measurement. This information was obtained via a university-based, geriatric, primary-care facility's medical records.[19] Fifty-three people met criteria for the vitamin $B_{12}$ deficient group and a set of controls were matched within one year of age and sex, in a ratio of 4:1, with 212 people constituting the normal-status control group.[19] Overall, chronic, defined as greater than 12 months, and current use of $H_2$RA and/or PPI was associated with significantly increased risk of vitamin $B_{12}$ deficiency.[19] It was also found that multivitamin use

did not protect against deficiency of vitamin $B_{12}$.[19] Dharmarajan et al. found similar results and both Valuck and Ruscin and Dharmarajan et al. recommend screening of elderly individuals to reduce any complications that may arise due to untreated vitamin $B_{12}$ deficiency.[18,19] Valuck and Ruscin did not differentiate between $H_2$RA use and PPI use and recent studies have indicated that $H_2$RAs do not have an effect on vitamin $B_{12}$ levels.[19]

A 2002 case study followed a 78-year-old woman using $H_2$RAs and PPIs long term (4.5 years) for treatment of GERD.[42] Serum levels of vitamin $B_{12}$ were adequate at baseline and the patient developed a vitamin $B_{12}$ deficiency after prolonged use of both $H_2$RA and PPIs, although the vitamin $B_{12}$ deficiency was not shown until after use of PPIs.[42] When supplemented with 1000 µg/day, vitamin $B_{12}$ status improved, although sMMA and homocysteine did remain slightly elevated in the patient.[42] This improvement of vitamin $B_{12}$ levels indicates that malabsorption of food-bound $B_{12}$ occurred, not lack of IF.[42] Overall, Ruscin et al. concluded that vitamin $B_{12}$ deficiency should be considered in long-term use of PPIs, especially in people with poor nutritional intake.

Two 1994 studies analyzed vitamin $B_{12}$ absorption with omeprazole or hypochlorhydric states.[21,43] These studies both examined short-term use of omeprazole. Marcuard et al. provided either 20 mg or 40 mg of omeprazole daily for two weeks to participants and Saltzman et al. provided 40 mg of omeprazole daily for a minimum of 7 days.[21,43] Both studies found that omeprazole treatment did in fact impair protein-bound vitamin $B_{12}$ absorption, but Saltzman et al. observed that the Schilling tests using crystalline vitamin $B_{12}$ were normal among all groups (i.e. control group, omeprazole treatment, and atrophic gastritis group).[21] A Schilling test is used to identify if vitamin $B_{12}$ is absorbed normally.[21,43] Marcuard et al. stated that omeprazole therapy causes a dose-dependent decrease in absorption of vitamin $B_{12}$.[43] Saltzman et al. concluded that

this impairment was similar to what occurs in atrophic gastritis.[21] Both studies are limited by small sample size with 10 participants and 19 participants, respectively. The age groups examined also differed, with Marcuard et al. examining 10 healthy male volunteers 22-50 years old while Saltzman et al. examined healthy, free-living elderly individuals >60 years old.[21,43] Saltzman et al. also examined if an acidic beverage ingested with protein-bound vitamin $B_{12}$ would increase absorption of vitamin $B_{12}$.[21] It was found that cranberry juice and a diluted $HCl^-$ solution did indeed increase the absorption of protein bound vitamin $B_{12}$, although more research is needed to confirm if this finding was of clinical significance.[21]

A 1992 study aimed to examine the effect of prolonged omeprazole therapy on absorption of micronutrients including vitamin $B_{12}$.[44] The participants had been taking omeprazole for 6-48 months primarily for GERD at a dosage of primarily 20 mg/day.[44] Although a downward trend regarding vitamin $B_{12}$ status was observed after 3 years of treatment, this was not statistically significant.[44]

To summarize, many studies support the hypothesis that long-term use of PPIs (at least 1 year) may be associated with low serum vitamin $B_{12}$ levels although the etiology is not completely understood. Most studies examined serum vitamin $B_{12}$,with newer research utilizing MMA and homocysteine in conjunction with serum vitamin $B_{12}$ due to the increased sensitivity of these biomarkers. No studies have investigated uMMA as a biomarker in older adult patients utilizing PPIs.

## Conclusion

The research shows that the elderly are more susceptible to vitamin $B_{12}$ deficiency due to age-related gastric changes and polypharmacy, although the population ages 50-65 are largely

ignored in the literature. Specifically, PPIs may exacerbate the gastric effects of chronic atrophic gastritis, which is commonly found in the aging population. Insufficient dietary intake is typically not the cause of a deficiency while malabsorption seems to be the main culprit. The biomarkers used to determine deficiency, such as serum vitamin $B_{12}$, are not as sensitive as uMMA and may underestimate the level of deficiency. Untreated vitamin $B_{12}$ deficiency may lead to worsened neurological symptoms which may lead to cognitive impairment and reduced quality of life. If a deficiency is not addressed in a timely manner, the neurological effects may be irreversible. It is worthwhile to add to the current body of research regarding the usefulness of uMMA and the importance of identifying vitamin deficiencies in a timely manner to prevent any deleterious effects in the aging population in order to protect this population and maintain optimal cognitive function and quality of life as this generation begins to approach advanced age.

# CHAPTER 3

# METHODS

## Selection of the Sample

A quasi-experimental design was used for this study. Thirty men and women between the ages of 50-70 years old were recruited using informational flyers posted in local assisted-living facilities, the university campus, and other community locations (Appendix A). Flyers were also posted via social media avenues and phone calls were made to recruit participants using a standardized recruitment script (Appendix B). A recruitment screening form was also utilized to assist with filtering eligible and non-eligible participants (Appendix C). Approval from the Institutional Review Board (IRB) and Institutional Biosafety Committee (IBC) of Northern Illinois University was obtained before recruitment of participants and data collection (Appendix D and E) and all subjects signed an informed consent prior to participating in this study (Appendix F).

Fifteen subjects were recruited based on daily use of PPIs for a minimum of one year. Fifteen subjects were then recruited based on no past or current use of PPIs and these participants were age- and gender-matched to subjects in the PPI group. The participants were age-matched within ±3 years. Exclusion criteria included history of Crohn's disease, ulcerative colitis, pernicious anemia, liver or kidney disease, use of metformin, adherence to a vegetarian or vegan

diet, or use of intramuscular shots of vitamin $B_{12}$ or nasal Nascobal (Strativa Pharmaceuticals, Spring Valley, NY).

## Data Collection

Data collection occurred at the nutrition laboratory in Wirtz Hall 308A at Northern Illinois University. Appointments were scheduled for participants based on availability and convenience of the participants. At the appointment, a consent form (Appendix F) was administered and signed by each subject, and subjects were instructed to bring a completed three-day food record (Appendix G). This three-day food record was mailed or hand delivered to participants before their appointment and questions were addressed regarding the food log at that time. A short survey was administered (Appendix H and I) and then anthropometrics were assessed followed by collection of a urine sample for uMMA assay.

## Instruments

### Survey

An informational survey was administered to participants to collect information regarding demographics, lifestyle behaviors, use of vitamin supplements and use of PPIs. Habits that were questioned included smoking habits, use of vitamin $B_{12}$, vitamin $B_6$ and folic acid supplementation, and use of specific PPIs (Appendix H and I). These surveys were analyzed by the researcher and responses were compiled and entered into the Statistical Package for Social Sciences (SPSS) for Windows (Version 21.0, 2013, SPSS, Inc, Chicago, IL).

## Diet Analyses

Nutrition Calc Plus (version 3.6.1 McGraw-Hill Companies, Columbus, OH) was utilized to assess each participant's three-day food record (Appendix G). Analyses performed included the following: total Kcal/kg; fat, protein and carbohydrate as percentages of total Kcal/kg; and dietary vitamin $B_{12}$, vitamin $B_6$ and folic acid. The diet analyses were completed by the researcher to allow for consistency when interpreting information provided from the three-day food records.

## Anthropometrics

Participants had anthropometric measures completed in lightweight clothing and bare feet. Height was obtained to the nearest half inch at the time of the appointment using a wall-mounted stadiometer (Ayrton S-100, Prior lake, MN). Weight, fat mass, body fat percent, fat free mass and body mass index (BMI) was assessed utilizing a bioelectrical impedance scale (InBody 520, Biospace Inc., Los Angeles, CA). BMI was calculated by the InBody 520 using the standard equation (kilogram per meter squared).

## Urine Samples

Urine samples were collected in sterile containers and transferred to vials containing 5 mg of thymol as a preservative, allowing samples to be mailed unrefrigerated. The vials were stored at -20ºC until all data had been collected. The samples were then shipped overnight to Norman Clinical Laboratory, Inc. (Cincinnati, Ohio) for analysis.

## Laboratory Measurements

The urine samples were analyzed by Norman Clinical Laboratories Inc. (Cincinnati, Ohio). Urine levels of MMA were determined using an ion-monitoring isotope dilution gas chromatography mass spectrometry (GC/MS). uMMA levels were normalized to urinary creatinine levels. Five hundred ng of deuterated MMA as an internal standard and a gas chromatograph (Varian 3400, Varian Associates, Sugarland, TX) equipped with a capillary column (30-m, DB-5, 0.25-um film thickness, 0.5 mm inner diameter, J&W Scientific Co., Folsom, CA) were interfaced to a mass spectrometer (Finnigan MAT 800 ion trap detector, San Jose, CA). The GC/MS was equipped with a Finnigan MAT A200S autosampler. The GC was programmed from 140º to 225ºC at 4.7ºC/min and then to 280ºC at 20ºC/min with a 10-minute hold time. For the data analysis, levels of uMMA are considered to be normal if they were <3.8 µg MMA/mg creatinine or <3.6 mmol/mol creatinine.[12]

## Data Analysis

Due to the small sample size and the matched-pairs design, this study used the non-parametric related-samples Wilcoxon signed rank test to determine differences in key variables between PPI users and non-PPI users.[12,45] This test was also used to test the hypothesis that subjects using PPIs would have higher uMMA levels than their age- and gender-matched controls not using PPIs.[12,45]

The mean and standard deviation were determined for each key variable between PPI users and non-PPI users and provided as M ± SD. The median was also provided for each key variable. The tests for equivalency between key variables of each group was provided as a *Z*-

value. The testing of the hypothesis was also reported as a *Z*-value. Statistical significance for all data analysis was accepted at the $p<0.05$ level of confidence. Data was analyzed by using the Statistical Package for Social Sciences (SPSS) for Windows (Version 21.0, 2013, SPSS, Inc., Chicago, IL).

# CHAPTER 4

# RESULTS

## Research Methodology

The purpose of this study was to determine if uMMA increases with chronic use of PPIs. First, PPI users were recruited and then gender-matched and age-matched (±3 years) with non-PPI users. Participants were required to provide a urine sample in order to evaluate uMMA. Participants were also required to complete a three-day food record for nutritional analysis, complete a survey regarding demographic and lifestyle habits, and consent to collection of anthropometric data.

## Participants

### General Characteristics

The participants ranged in age from 50-68 years old with a mean age of 56.6 years old. Participants were gender-matched and age-matched within ±3 years. Ten males and 20 females participated in the study. The mean BMI for the PPI users was 33.2 and the mean BMI for the non-PPI users was 28.3, which was significantly different ($Z$=-2.33, $p$=0.02). Most participants were Caucasian (93.3%, n=28) while 6.7% of the participants were African American (n=2).

## Physical Activity

A third of participants reported no structured physical activity (33.3%, n=10) while 20% (n=6) reported structured physical activity 5 days per week. Twelve participants reported structured physical activity ranging from 2-4 days per week (13.3%, n=4) while 3.3% (n=1) of participants reported structured physical activity both 1 day per week and 7 days per week. Of those participants who exercised, 20.0% (n=6) reported length to be approximately 30-39 minutes and only 6.7% (n=2) reported exercise to last 60 minutes or longer. Most exercising participants (40.0%, n=12) reported physical activity to be moderate intensity and 20.0% (n=6) reported low intensity exercise.

## Details Regarding PPI Use

The PPI users had been taking PPIs for a range of 1.5-15 years with a mean of 7 years (M(SD)= 7.0±3.9, Mdn=7.0). Two participants reported taking PPIs over-the-counter (13.3%, n=2), nine participants reported taking PPIs under doctor's orders (60%, n=9), and the remaining four participants reported taking PPIs over-the-counter with a doctor's guidance (33.3%, n=4). Acid reflux was the most common reason for PPI prescription with 86.7% (n=13) of PPI users reporting acid reflux or GERD as the reason for PPI use. The remaining two PPI users reported use of PPIs for a hiatal hernia and gastritis.

## Equivalency of Groups

### Physical Characteristics

The PPI users and non-PPI users were tested for equivalency using the related-samples Wilcoxon signed rank test. The PPI users and non-PPI users were significantly different regarding BMI ($Z$= -2.33, $p$=0.02), body fat percent ($Z$=-2.36, $p$=0.02), and body fat mass ($Z$= -2.33, $p$=0.02). PPI users, on the average, had a higher BMI, body fat percent, and body fat mass. Despite these differences, the groups were not significantly different in age ($Z$= -0.11, $p$=0.9) or lean body mass ($Z$= -0.91, $p$= 0.36). Table 1 provides the mean, median and significance level of the physical characteristics of PPI users and non-PPI users and Figure 3 displays the mean for each physical characteristic for PPI users and non-PPI users.

Table 1

Physical Characteristics of PPI Users and Non-PPI Users

| | **PPI Users (n=15)** | | **Non-PPI Users (n=15)** | | |
|---|---|---|---|---|---|
| | **Mean±SD** | **Median** | **Mean±SD** | **Median** | ***P* Value** |
| Age | 56.6±5.5 | 56.0 | 56.5±5.2 | 54.0 | 0.91 |
| BMI | 33.2±6.6 | 30.9 | 28.3±4.4 | 27.3 | 0.02* |
| Body Fat Percent (%) | 40.4±8.2 | 39.8 | 35.1±8.0 | 35.6 | 0.02* |
| Body Fat Mass (kg) | 38.3±14.3 | 33.1 | 28.6±9.6 | 26.3 | 0.02* |
| Lean Body Mass (kg) | 54.7±10.3 | 53.0 | 52.0±10.5 | 51.7 | 0.36 |

**Note:** P values results from related-samples Wilcoxon signed rank tests.

* indicates $p<0.05$

SD = Standard deviation

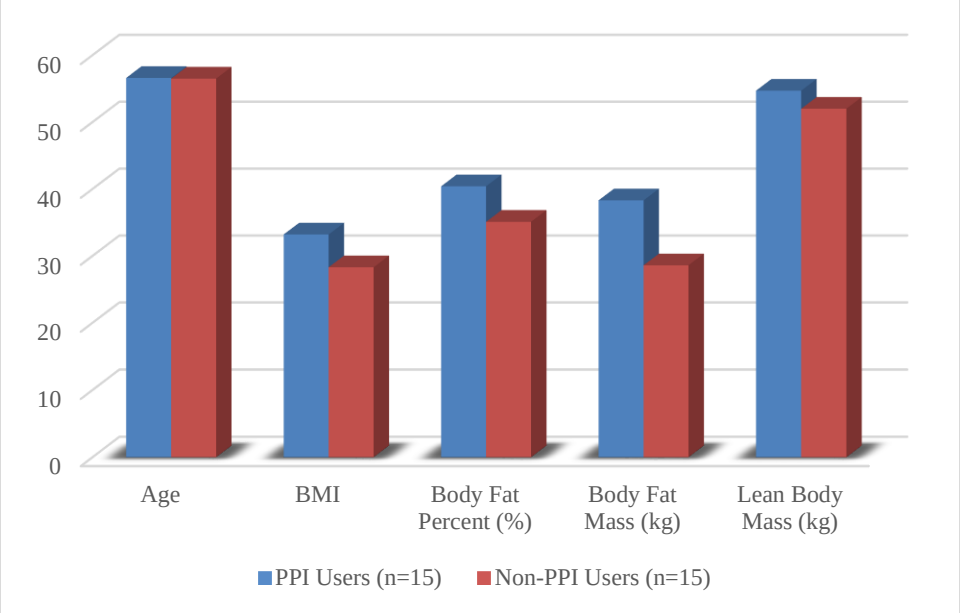


Figure 3: Physical characteristics of PPI users and non-PPI users

## Dietary Intake

Dietary intake data was not available for one non-PPI user participant; therefore, the data presented for dietary intake reflects the related-samples Wilcoxon signed rank test using 14 pairs. Dietary intake descriptive values were calculated with 29 participants and equivalency of group regarding dietary intake was analyzed using 14 vs. 15 pairs. Most participants reported having an excellent appetite, 63.3% (n=19), while 26.7% (n=8) reported a good appetite and 10.0% (n=3) reported a fair appetite. Tests for equivalency of groups indicated no significant differences between average daily caloric intake (kcal/kg) ($Z$=0.09, $p$=0.93), carbohydrates as percentage of caloric intake ($Z$=0.03, $p$=0.98), protein as percentage of caloric intake ($Z$=-1.41, $p$=0.16), fat as percentage of caloric intake ($Z$=1.73, $p$=0.08), dietary vitamin $B_{12}$ (µg) ($Z$=-1.15, $p$=0.25), dietary vitamin $B_6$ (mg) ($Z$=-1.29, $p$=0.20), and dietary folate (µg) ($Z$=-0.66, $p$=0.51). Table 2 provides the mean, median and significance level of dietary intake for PPI users and non-PPI users. Figure

4 displays the mean for all dietary intake except dietary folate for PPI users and non-PPI users and Figure 5 displays the mean for dietary folate intake for PPI users and non-PPI users.

Table 2

Dietary Intake of PPI Users and Non-PPI Users

| | PPI Users (n=15) | | Non-PPI Users (n=15) | | |
|---|---|---|---|---|---|
| | **Mean±SD** | **Median** | **Mean±SD** | **Median** | ***P* Value** |
| Average Daily Caloric Intake (kcal/kg) | 19.3±6.7 | 18.1 | 20.0±4.7 | 20.4 | 0.93 |
| Carbohydrate as % of Caloric Intake | 40.9±6.3 | 39.7 | 41.2±5.5 | 40.8 | 0.98 |
| Protein as % of Caloric Intake | 20.6±4.2 | 19.5 | 18.2±3.3 | 18.0 | 0.16 |
| Fat as % of Caloric Intake | 36.7±4.7 | 36.3 | 38.8±4.8 | 38.2 | 0.08 |
| Dietary Vitamin $B_{12}$ (µg) | 3.8±3.1 | 2.9 | 2.7±1.8 | 2.0 | 0.25 |
| Dietary Vitamin $B_6$ (mg) | 1.1±0.7 | 0.8 | 1.1±0.8 | 0.8 | 0.20 |
| Dietary Folate (µg) | 262.0±195.3 | 185.2 | 178.0±105.3 | 173.2 | 0.51 |

**Note:** P values results from related-samples Wilcoxon signed rank tests. Total of percentages for carbohydrates, protein, and fat may not equal 100% due to rounding.
SD = Standard deviation

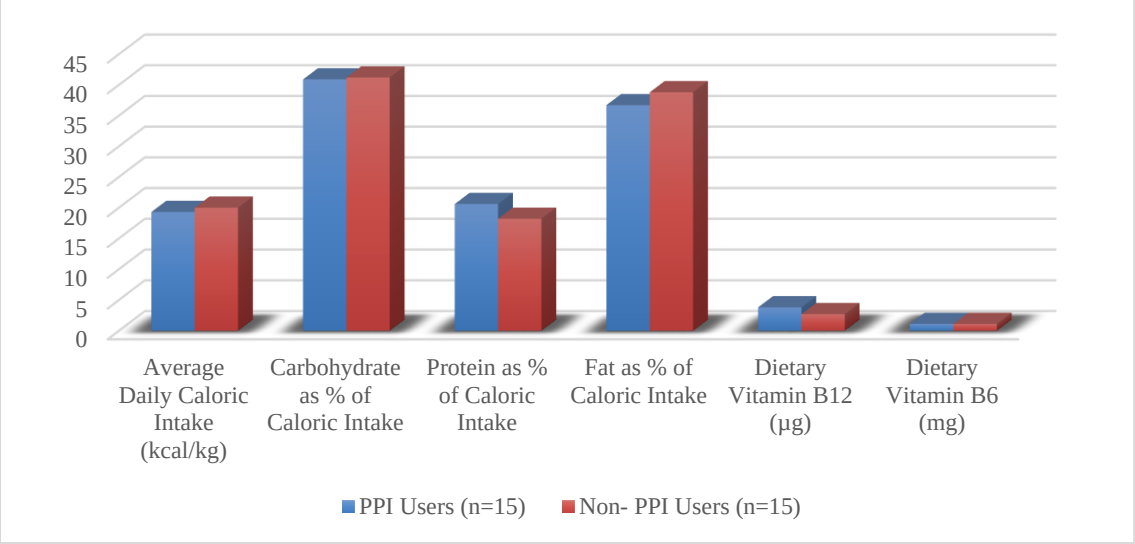


Figure 4: Dietary intake of PPI users and non-PPI users

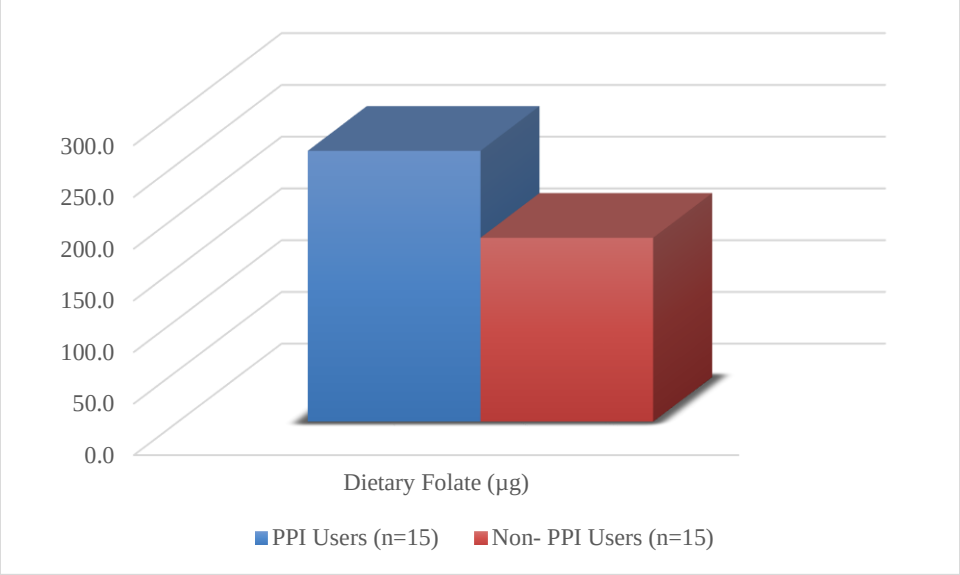


Figure 5: Dietary intake of folate of PPI users and non-PPI users

## Supplemental Intake

Tests for equivalency of groups regarding supplemental intake indicated no significant difference between supplemental vitamin $B_{12}$ (µg) ($Z$= -0.23, $p$=0.82), supplemental vitamin $B_6$ (mg) ($Z$= -0.09, $p$=0.93), and supplemental folic acid (µg) ($Z$= 0.00, $p$=1.00). Only six PPI users did not consume a multivitamin while the remaining PPI users consumed a multivitamin with vitamin $B_{12}$, vitamin $B_6$, and folic acid with one PPI user taking a super vitamin B-complex daily. Eight non-PPI users reported not using a multivitamin while the remaining seven non-PPI users consumed a multivitamin daily. Table 3 provides the mean, median and significance level of supplemental B vitamin intake. Figure 6 displays the mean supplemental intake of vitamins $B_{12}$ and $B_6$ and Figure 7 displays the mean supplemental intake of folic acid.

Table 3

Supplemental Intake of PPI Users and Non-PPI Users

| | PPI Users (n=15) | | Non-PPI Users (n=15) | | |
|---|---|---|---|---|---|
| | **Mean±SD** | **Median** | **Mean±SD** | **Median** | ***P* Value** |
| Supplemental Vitamin $B_{12}$ (µg) | 25.8±35.3 | 15 | 64.1±204.1 | 0.0 | 0.82 |
| Supplemental Vitamin $B_6$ (mg) | 3.0±2.8 | 3 | 3.7±6.5 | 0.0 | 0.93 |
| Supplemental Folic Acid (µg) | 246.7±216.7 | 400 | 293.3±439.9 | 0.0 | 1.00 |

**Note:** P values results from related-samples Wilcoxon signed rank tests.

SD = Standard deviation

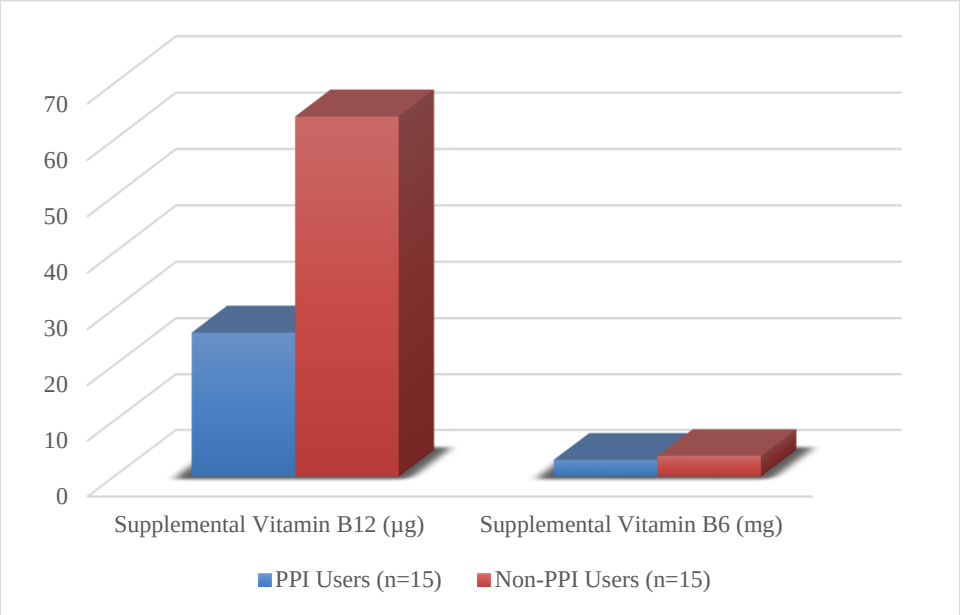


Figure 6: Supplemental intake of PPI users and non-PPI users

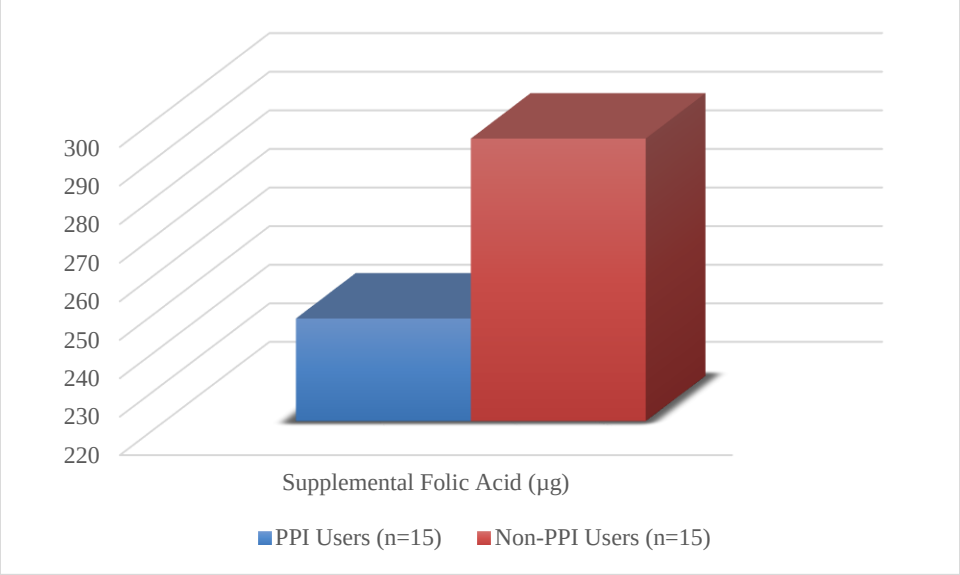


Figure 7: Supplemental intake of folic acid of PPI users and non-PPI users

## Urinary Methylmalonic Acid

uMMA ranged from 0.90 to 2.80 µg/mg creatinine among the PPI users and 1.20 to 3.30 µg/mg creatinine among the non-PPI users. The mean uMMA for PPI users was 1.6 µg/mg creatinine (M(SD)= 1.6±0.6, Mdn=1.6) while the mean uMMA for the control group was 2.0 µg/mg creatinine (M(SD)=2.0±0.6, Mdn=1.8). The hypothesis that PPI users would have higher uMMA levels than their gender-matched and age-matched controls was not supported ($Z$=1.54, $p$=0.12). Table 4 provides the mean, median and significance level of uMMA and Figure 8 displays the mean uMMA for PPI users and non-PPI users.

Table 4

Urinary Methylmalonic Acid Levels of PPI Users and Non-PPI Users

| | PPI Users (n=15) | | Non-PPI Users (n=15) | | |
|---|---|---|---|---|---|
| | **Mean±SD** | **Median** | **Mean±SD** | **Median** | ***P* Value** |
| uMMA Levels (µg uMMA/mg creatinine) | 1.6±0.6 | 1.6 | 2.0±0.6 | 1.8 | 0.12 |

**Note:** P values results from related-samples Wilcoxon signed rank tests.

SD = Standard deviation

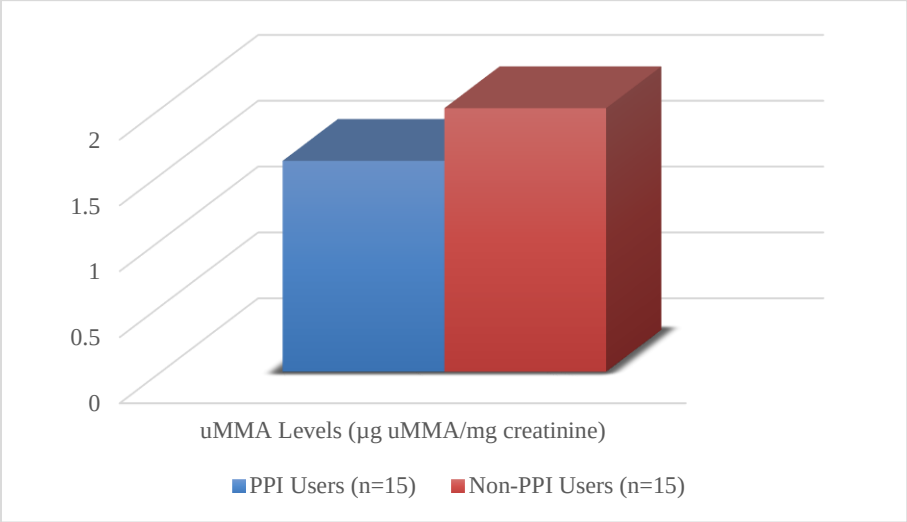


Figure 8: Urinary methylmalonic acid levels of PPI users and non-PPI users

# CHAPTER 5

# DISCUSSION

This study shows for the first time that there were no differences in uMMA levels between those taking PPIs versus those not taking PPIs in the 50-70-year-old age range. The findings of this study concur with those of Lukaszuk et al., who evaluated uMMA levels in 22-50-year-old adults.[12] Two additional studies conducted among adults in their mid-fifties and those who were >65 years old also found that taking PPIs did not deleteriously affect serum vitamin $B_{12}$ levels.[15,16] In direct contrast to the findings of these studies, three other studies found that PPI use of at least one year negatively affected serum vitamin $B_{12}$ status; however, the average age of the subjects in those studies was 80-82 years old, much older than the average age of the subjects in this study, which was 57 years old.[17-19]

Ruscin et al. recommended monitoring of vitamin $B_{12}$ status in individuals taking PPIs for more than four years because PPIs may reduce vitamin $B_{12}$ absorption.[42] In this current study, 10 of 15 subjects had been taking PPIs for more than 5 years and 7 of 15 subjects had been taking PPIs for more than 7 years, yet their uMMA levels remained within a normal range. The study results seem to indicate that PPI users recruited for this study had GERD but were otherwise healthy and likely had normal vitamin $B_{12}$ stores before being placed on PPIs. Thus, monitoring may still be recommended for those at higher risk of vitamin $B_{12}$ deficiency.

Vitamin $B_{12}$ is stored and endogenously recycled via the liver.[23,25] As stated previously, a deficiency of vitamin $B_{12}$ would take a long length of time to develop, approximately 3-5 years.[23,25] The PPI users in this study had been taking PPIs for an average of 7 years, yet their uMMA levels indicated that tissue stores of vitamin $B_{12}$ remained within a normal range.

## Physical Characteristics

PPI users and non-PPI users were significantly different regarding BMI, body fat percent, and body fat mass, with PPI users displaying a higher BMI, body fat percent, and body fat mass than non-PPI users. The BMI of PPI users (M(SD) = 33.2±6.6, Mdn = 30.9) is classified as obese while the BMI of non-PPI users (M(SD) = 28.3±4.4, Mdn = 27.3) is classified as overweight.[46] Because there is an association between BMI and GERD, obesity is commonly seen as a risk factor contributing to the development of GERD.[47,48] Research has indicated that obesity is associated with a significant 1.5 to 2 times increase in risk of GERD development and this association has been confirmed utilizing a multichannel intraluminal impedance pH monitoring test.[49-51] The mechanism of this relationship is largely unknown; however, one study suggests that obesity is associated with a post-prandial transient lower esophageal sphincter relaxation with subsequent acid reflux.[48] Subjects with a higher BMI in the aforementioned study were more likely to have acid reflux and therefore had a greater reliance on PPIs.

## Dietary and Supplemental Intake

PPI users and non-PPI users were not significantly different regarding dietary or supplemental intake of B vitamins. When dietary and supplemental intakes were combined, the RDA for vitamin $B_{12,}$ vitamin $B_{6,}$ and folic acid was met. The only vitamin that would affect

uMMA levels is vitamin $B_{12}$, and all subjects were ingesting a sufficient amount of vitamin $B_{12}$ based on the established RDA. There is no reason to suggest that dietary intake or supplementation had any effect on uMMA results.

## Urinary Methylmalonic Acid

The only other study using uMMA to measure vitamin $B_{12}$ status was conducted by Lukaszuk et al., which investigated the effect of PPI use on vitamin $B_{12}$ status in people 22-50 years old. This study and the current study both found that uMMA was not significantly different between PPI users and non-PPI users.[12] The use of uMMA as a biomarker of vitamin $B_{12}$ status has been confirmed by various studies.[11] No participants were approaching the abnormal level of uMMA of > 3.8 µg MMA/mg creatinine.[12]

## Conclusion

The results of this study indicate that chronic use of PPIs in people 50-70 years old recruited for this study did not negatively affect vitamin $B_{12}$ status as assessed by uMMA levels. The participants in this study were relatively healthy (other than GERD) with no history of Crohn's disease, ulcerative colitis, pernicious anemia, and liver or kidney disease. Future studies should recruit a larger sample size and target higher risk populations such as those individuals with malabsorption disorders, liver disease, renal disease, vegans or individuals following a gluten-free diet. One may also consider recruiting individuals with chronic atrophic gastritis or individuals over the age of 70.

# CHAPTER 6

# LIMITATIONS AND FUTURE RESEARCH

## Limitations

The current research was limited by a small sample size (n=30). To accommodate the small sample size, a nonparametric test was required to be utilized, which is not as powerful as a parametric test such as the paired-samples *t*-test. Although more recent research has shown the justification for using the *t*-test in small sample sizes, the variable being tested must be normally distributed.[52] This was not the case with the uMMA levels among PPI users and non-PPI users, thus eliminating the ability to use a parametric test.

The PPI users and non-PPI users were also significantly different in regards to three physical characteristics. The groups were significantly different regarding BMI, body fat percent, and body fat mass. Ideally, the groups would not be significantly different regarding physical characteristics. However, the ages of participants were not significantly different between PPI users and non-PPI users. PPI users were gender- and age-matched within ± 3 years, although ideally, participants would be age-matched to exactly the same age. Due to difficulty recruiting participants, this was not feasible and the researchers determined it was appropriate to match age as closely as possible.

The current research was also cross-sectional in nature. Researchers were unable to obtain uMMA levels prior to PPI use to analyze any affect after long-term use of PPIs. This was not feasible due to time and monetary constraints.

## Future Research

A larger sample size would be beneficial for future research to contribute to stronger statistical power. Studies may be more beneficial in vulnerable populations such as the elderly or individuals with several co-morbidities. Studies may also be focused towards greater length of PPI use and evaluation of vitamin $B_{12}$ status; however, the average length of PPI use of the current research was 7 years, which is quite long term.

REFERENCES


1. Administration on Aging. Administration for Community Living. US Department of Health and Human Services. Profile of Older Americans: 2013. Administration of Community Living Web site. http://www.aoa.gov/AoARoot/Aging_Statistics/Profile/2013/3.aspx. Published 2013. Accessed July 17. 2014.

2. Achem SR. DeVault KR. Gastroesophageal reflux disease and the elderly. *Gastroenterol Clin N Am.* 2014;43:147-160.

3. Yang Y, Metz DC. Safety of proton pump inhibitor exposure. *Gastroenterology*. 2010;139:1115-1127.

4. Masclee GMC, Sturkenboom MCJM, Kuipers EJ. A benefit-risk assessment of the use of proton pump inhibitors in the elderly. *Drugs Aging*. 2014;31:263-282.

5. Saltzman JR, Russell RM. The Aging gut: nutritional issues. *Gastroenterol Clin North Am*. 1998;27(2):309-324.

6. Allen, LH. How common is vitamin-$B_{12}$ deficiency? *Am J Clin Nutr.* 2009;89(suppl):S693-S696.

7. Pfeiffer CM, Caudill SP, Gunter EW, et al. Biochemical indicators of B vitamin status in the US population after folic acid fortification results from the National Health and Nutrition Examination Survey 1999-2000. *Am J Clin Nutr.* 2005;82:442-450.

8. Wolters M, Strohle A, Hahn A. Cobalamin: a critical vitamin in the elderly. *ISRN Prev Med.* 2004;39:1256-1266.

9. Higdon J, Drake VJ, Delage B, et al. Vitamin $B_{12}$. Linus Pauling Institute: Micronutrient Information Center Web site. http://lpi.oregonstate.edu/infocenter/vitamins/vitaminB12/. Published 2000. Updated April 2014. Updated June 4, 2015. Accessed July 2, 2014.

10. Chatthanawaree W. Biomarkers of cobalamin (vitamin $B_{12}$) deficiency and its application. *J Nutr Health Aging*. 2011;15(3):227-231.

11. Hill MH, Flatley JE, Barker ME, et al. A vitamin B-12 supplement of 500 µg/d for eight weeks does not normalize urinary methylmalonic acid or other biomarkers of vitamin B-12 status in elderly people with moderately poor vitamin B-12 status. *J Nutr.* 2013;143:142-147.

12. Lukaszuk JM, Prawitz AD, Shokrani M, et al. Methylmalonic acid levels in non-elderly adult chronic PPI users. *J Hum Nutr Food Sci.* 2013;1:1004.

13. Grober U, Kisters K, Schmidt J. Neuroenhancement with vitamin $B_{12}$ – underestimated neurological significance. *Nutrients*. 2013;5:5031-5045.

14. Gultepe M, Ozcan O, Avsar K, et al. Urine methylmalonic acid measurements for the assessment of cobalamin deficiency related to neuropsychiatric disorders. *Clin Biochem.* 2003;36:275-282.

15. Hirschowitz BI, Worthington J, Mohnen J. Vitamin $B_{12}$ deficiency in hypersecretors during long-term acid suppression with proton pump inhibitors. *Aliment Pharmacol Ther*. 2008;27:1110-1121.

16. Den Elzen WPJ, Groeneveld Y, De Ruijter W, et al. Long-term use of proton pump inhibitors and vitamin B12 status in elderly individuals. *Aliment Pharmacol Ther.* 2008;27:491-497.

17. Rozgony NR, Fang C, Kuczmarski MF. Vitamin $B_{12}$ deficiency is linked with long-term use of proton pump inhibitors in institutionalized older adults: could a cyanocobalamin nasal spray be beneficial. *J Nutr Elder*. 2010;29(1):87-99.

18. Dharmarajan TS, Kanagala MR, Murakonda P, et al. Do acid-lowering agents affect vitamin $B_{12}$ status in older adults? *J Am Med Dir Assoc.* 2008;9:162-167.

19. Valuck RJ, Ruscin JM. A case-control study on adverse effects: $H_2$ blocker or proton pump inhibitor use and risk of vitamin $B_{12}$ deficiency in older adults. *J Clin Epidemiol.* 2004;57:422-428.

20. Ito T, Jensen RT. Association of long-term proton pump inhibitor therapy with bone fractures and effects on absorption of calcium, vitamin $B_{12}$, iron, and magnesium. *Curr Gastroenterol Rep*. 2010;12(6):448-457.

21. Saltzman JR, Kemp JA, Golner BB, et al. Effect of hypochlorhydria due to omeprazole treatment or atrophic gastritis on protein-based vitamin $B_{12}$ absorption. *J Am Coll Nutr.* 1994;13(6):584-591.

22. Abraham NS. Proton pump inhibitors: potential adverse effects. *Curr Opin Gastroenterol*. 2012;28:615-620.

23. Gropper SS, Smith JL. *Advanced Nutrition and Human Metabolism*. 6th ed. United States: Wadsworth Cengage Learning; 2013: 344-360.

24. Calabresi PA. Multiple sclerosis and demyelinating conditions of the central nervous system. In: Goldman L, Schafer AI, ed. *Cecil Medicine*. 24th ed. Philadelphia, Pa: Saunders Elsevier; 2011.

25. Howden CW. Vitamin $B_{12}$ levels during prolonged treatment with proton pump inhibitors. *J Clin Gastroenterol.* 2000;30(1):29-33.

26. vanAsselt DZB, de Groot LCPGM, van Staveren WA, et al. Role of cobalamin intake and atrophic gastritis in mild cobalamin deficiency in older Dutch subjects. *Am J Clin Nutr.* 1998;68:328-334.

27. Carmel, R. Cobalamin, the stomach, and aging. *Am J Clin Nutr.* 1997;66:750-759.

28. Carmel, R. Diagnosis and management of clinical and subclinical cobalamin deficiencies: why controversies persist in the age of sensitive metabolic testing. *Biochimie.* 2013;95:1047-1055.

29. Solfrizzi V, Panza, F, Capurso A. The role of diet in cognitive decline. *J Neurol Transm.* 2003;110:95-110.

30. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med.* 2002;346:476-483.

31. Norman, EJ. Urinary methylmalonic acid test may have greater value than the total homocysteine assay for screening elderly individuals for cobalamin deficiency. *Clin Chem.* 2004;50(8):1482.

32. Norman EJ, Morrison JA. Screening elderly populations for cobalamin (vitamin $B_{12}$) deficiency using the urinary methylmalonic acid assay by gas chromatography mass spectrometry. *Am J Med.* 1993;94:589-594.

33. Vogeser M, Muller W, Lorenzi S. External quality assessment of urinary methylmalonic acid quantification – results of a pilot study. *Clin Chem Lab Med.* 2007;45(5):695-696.

34. Lindenbaum J, Rosenberg IH, Wilson PWF, et al. Prevalence of cobalamin deficiency in the Framingham elderly population. *Am J Clin Nutr*. 1994;60:2-11.

35. Sipponen P, Laxen F, Huotari K, et al. Prevalence of low vitamin $B_{12}$ and high homocysteine in serum in an elderly male population: Association with atrophic gastritis and *Helicobacter pylori* infection. *Scand J Gasatroenterol*. 2003;12:1209-1216.

36. Mirkazemi C, Peterson GM, Tenni PC, et al. Vitamin $B_{12}$ deficiency in Australian residential aged care facilities. *J Nutr Health Aging.* 2012;16(3):277-280.

37. Loikas, S, Koskinen P, Irjala K, et al. Vitamin $B_{12}$ deficiency in the aged: a population-based study. *Age and Ageing.* 2007;36:177-183.

38. Gill JM, Player MS, Metz DC. Balancing the risks and benefits of proton pump inhibitors. *Ann Fam Med.* 2011;9:200-202.

39. Heidelbaugh JJ, Goldberg KL, Inadomi JM. Overutilization of proton pump inhibitors: a review of cost-effectiveness and risk in PPI. *Am J Gastroenterol*. 2009;104:S27-S32.

40. Bavishi C, DuPont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Aliment Pharmacol Ther.* 2011;34:1269-1281.

41. Thomson ABR, Sauve MD, Kassam N, et al. Safety of the long-term use of proton pump inhibitors. *World J Gastroenterol.* 2010;16(19):2323-2330.

42. Ruscin JM, Page II RL, Valuck RJ. Vitamin $B_{12}$ deficiency associated with histamine$_2$-receptor antagonists and a proton pump inhibitor. *Ann Pharmacother*. 2002;36:812-816.

43. Marcuard SP, Albernaz L, Khazanie PG. Omeprazole therapy causes malabsorption of cyanocobalamin (Vitamin $B_{12}$). *Ann Intern Med.* 1994;120:211-215.

44. Koop H, Bachem MG. Serum iron, ferritin, and vitamin $B_{12}$ during prolonged omeprazole therapy. *J Clin Gastroenterol.* 1992;14(4):288-292.

45. Harris JE, Boushey C, Bruemmer B, et al. Publishing nutrition research: a review of nonparametric methods, part 3. *J Am Diet Assoc.* 2008;108:1488-1496.

46. Charney P, Malone AM. *ADA Pocket Guide to Nutrition Assessment*. Chicago, IL: Academy of Nutrition and Dietetics; 2009: 156-157.

47. Corley DA, Kubo A. Body mass index and gastroesophageal reflux disease: a systematic review and meta-analysis. *Am J Gastroenterol.* 2006;108:2619-2628.

48. Wu JC, Mui L, Cheung CM, et al. Obesity is associated with increased transient lower esophageal sphincter relaxation. *Gastroenterology.* 2007;132:883-889.

49. El-Serang H, Graham DY, Satia JA, et al. Obesity is an independent risk factor for GERD symptoms and erosive esophagitis. *Am J Gastroenterol.* 2005;100:1243-1250.

50. El-Serang H. The Association between obesity and GERD: a review of the epidemiological evidence. *Dig Dis Sci.* 2008;53:2307-2312.

51. Hajar N, Castell DO, Ghomrawi H, et al. Impedance pH confirms the relationship between GERD and BMI. *Dig Dis Sci.* 2012;57:1875-1879.

52. deWinter JCF. Using the student's *t*-test with extremely small sample sizes. *Practical Assessment, Research & Evaluation.* 2013;18(10):1-12.

# APPENDIX A

# RECRUITMENT FLYER


# Are you currently taking Prilosec, Zegerid, Prevacid, Aciphex, Protonix or Nexium?

Participants needed for a study to determine the effects of proton pump inhibitors (PPI), which may reduce stomach acid production, on urinary vitamin B12 levels.

Requirements:

- Individuals ages 50 to 90 years old
- Must be taking one of the above medications to reduce stomach acid production for at least 1 year (generic name ends in "prazole")
- No history of Crohn's disease, ulcerative colitis, pernicious anemia, liver or kidney disease or following a vegetarian diet
- No use of the medication, metformin
- No electronic implants i.e. pacemakers
- Cannot be using the nasal B12 supplement, Nascobal or intramuscular shots of vitamin B12

Participants will need to...

- Step on a scale to have their body composition evaluated i.e. weight, body fat %, muscle mass
- Keep a three day food record

Time Commitment: One 30 Minute Visit!

Participation is Free

Participants will receive a free nutrient analysis and their vitamin B12 status!

Northern Illinois University
**College of Health and Human Sciences**
Family, Consumer, and Nutrition Sciences
*Nutrition and Dietetics*

**Please Take One!! Call or Email for Questions!! Thank you!!!**

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## Are you currently **NOT** taking Prilosec, Zegerid, Prevacid, Aciphex, Protonix or Nexium?

Participants needed for a study to determine the effects of proton pump inhibitors (PPI), which may reduce stomach acid production, on urinary vitamin B12 levels.

Requirements:

- 67 y/o female, 66 y/o male, 51 y/o female, 56 y/o male, 57 y/o female, 60 y/o male
- No history of PPI use (the drugs listed above)
- No history of Crohn's disease, ulcerative colitis, pernicious anemia, liver or kidney disease, no use of the medication metformin or following a vegetarian diet
- No electronic implants i.e. pacemakers
- Cannot be using the nasal B12 supplement, Nascobal, or intramuscular shots of vitamin B12

Participants will need to...

- Step on a scale to have their body composition evaluated i.e. weight, body fat %, muscle mass
- Keep a three day food record
- Provide a urine sample in order for B12 status to be measured

Time Commitment: One 30 Minute Visit!

Participation is Free

Participants will receive a free nutrient analysis and their vitamin B12 status!

Northern Illinois University
**College of Health and Human Sciences**
Family, Consumer, and Nutrition Sciences
*Nutrition and Dietetics*

**Please Take One!! Call or Email for Questions!! Thank you!!!**

Tashia Warner
Phone: (815) 474-7339
E-mail: b2ppistudy@yahoo.com

# APPENDIX B

# RECRUITMENT SCRIPT

**Script for Introductory Remarks**

**Project:** Methylmalonic Acid Levels in Older Adult Chronic PPI Users

**Researchers:** Dr. Lukaszuk, Dr. Umoren, Dr. Shokrani, and Tashia Warner

Hello. My name is _____. I'm calling on behalf of Dr. Lukaszuk and colleagues who are conducting a study on how the use of stomach acid lowering medications affect vitamin $B_{12}$ levels. The potential benefits to you include learning your current vitamin $B_{12}$ levels and having your lean body mass and body fat percentage measured free of charge. If you are interested in participating, I will ask you a few questions to determine eligibility and provide you with more detail about the procedures. Are you interested in participating in this study, and if so, may I ask you a few screening questions to determine if you are eligible for this study?

***If the response is no, participant is not interested.***

Thank you. I appreciate your time. Goodbye.

***If the response is yes, participant is interested.***

Thank you. [Proceed with screening questions.]

**Screening Questions**

Before we proceed, I have a few questions for you to determine your eligibility.

Are you aged between 55-90 years old?

***If response is yes, proceed to next question:***

***If response is no, participant is not eligible.***

Do you eat meat and other animal products?

***If response is yes, proceed to next question.***

***If response is no, participant is not eligible.***

Do you suffer from Crohn's disease, ulcerative colitis, pernicious anemia, liver disease or kidney disease?

***If response is no, proceed to next question.***

***If response is yes, participant is not eligible.***

Are you taking the medication, metformin?

***If response is no, proceed to next question.***

***If response is yes, participant is not eligible.***

Do you have any electronic implants, i.e. pacemakers?

***If response is no, proceed to next question.***

***If response is yes, participant is not eligible.***

Are you receiving any intramuscular shots of $B_{12}$ or using the nasal spray, Nascobal?

***If response is no, participant is eligible to participate, proceed to final questions.***

***If response is yes, participant is not eligible.***

*Participant is eligible to participant in the study. The final questions serve to assign the eligible participant to their respective groups.*

Have you been taking medication to reduce stomach acid production for at least 1 year?

Examples of these medications include:

- Omeprazole (Prilosec, Zegerid)
- Lansoprazole (Prevacid/Prevacid I.V./Prevacid 24h/Prevacid Solu-Tab, Zoton)
- Dexlansoprazole (Kapidex)
- Pantoprazole (Protonix/Protonix I.V.)
- Rabeprazole (Aciphex)
- Esomeprazole (Nexium/Nexium I.V.)

***If response is yes, participant is assigned to treatment group.***

***If response is no, participant is assigned to control group.***

***If not eligible to participate:***

Thank you for your interest and for taking the time to answer our screening questions. Unfortunately, you currently do not meet the requirements of this study. However, would you like us to contact you regarding participation in future studies?

***If no:*** Thank you again for your time. Goodbye.

***If yes:*** May I have your email address? Thank you again for your time. Goodbye.

***If yes and would like to participate:***

Great! We will send you a **three day food record**. Would you prefer receiving the **three day food record** by email or by mail? We will also set a date for you to visit NIU to have your anthropometric measurements and to supply a urine sample.

[Obtain email or mailing address.]

There are instructions on the **three day food record**, but I will discuss them briefly with you as well. We ask that you write down everything you eat and drink for three days. Remember to include the little things such as condiments, sugar, milk, etc. An example is the sugar and milk that is added to your coffee. Please try to approximate portion sizes of each item eaten as best you can. Do not change the way you usually eat and continue to eat as you normally would. If you have any questions, feel free to contact us via phone or email at any time. We have these dates and times available for you to schedule your appointment. What day and time would work best for you?

[Set date and confirm. Collect contact information from participant.]

At this appointment, you sign a consent form, complete a short survey, allow anthropometrics to be measured, and provide a urine sample.

Thanks again for your time and for your willingness to participate in this study. We will send your **three day food record** out today and we will see you [date]. Contact us if you have any questions or need to reschedule. Thank you!

# APPENDIX C

# RECRUITMENT SCREENING FORM

# Screening for Eligibility
## PPI/NON-PPI Users

***Please fill in the blanks and highlight YES or NO to indicate your choice. Email the completed form to b12ppistudy@yahoo.com.***

**NAME: _____**
**DATE OF BIRTH: _____**
**AGE: _____**
**SEX: _____**

- Crohn's Disease? YES or NO
- Ulcerative Colitis? YES or NO
- Pernicious Anemia? YES or NO
- Use of Metformin? YES or NO
- Liver Disease? YES or NO
- Kidney Disease? YES or NO
- Vegetarian Diet? YES or NO
- Use of $B_{12}$ Injections? YES or NO
- Use of $B_{12}$ Nasal Spray? Example: Nascobal YES or NO
- Implanted Device? Example: Pacemaker YES or NO
- Use of one of the following medications for at least 1 year? Prilosec, Zegerid, Prevacid, Kapidex, Protonix, Aciphex, Nexium or any generic drug that ends in –azole.

  YES or NO

***Thank you for completing this questionnaire! You will be contacted of your eligibility shortly.***

# APPENDIX D

# IRB APPLICATION AND APPROVAL LETTER

# Application for Institutional Review of Research
## INVOLVING HUMAN SUBJECTS

**Note:** Please complete this form thoroughly keeping in mind that the primary concern is the **potential risk** (economic, ethical, legal, physical, political, psychological/emotional, social, breach of confidentiality, or other) to the participants. Provide **copies** of all materials to be used in the investigation. The Institutional Review Board (IRB) must have enough information about the transactions with the participants to evaluate the risks of participation.

**Name(s) and employee ID for faculty, Z-ID for students**
Dr. Judith Lukaszuk (A111938), Dr. Josephine Umoren (A101184), Dr. Masih Shokrani (A135887), Tashia Warner (Z1621408)

**Status:** ☒ Faculty ☐ Graduate Student ☐ Undergraduate Student

**Department:**
Family, Consumer & Nutrition Sciences and Medical Lab Sciences Program

**Mailing Address (if not department):**
FCNS

**Phone:** 815-753-6352 **E-mail** jmlukaszuk@niu.edu

**Project Title:**
Methylmalonic Acid Levels in Older Adult Chronic Proton Pump Inhibitor Users

**Proposed Data Collection Start Date:** November 2014

**Note:** Unless the authorized departmental reviewer (e.g., chair or designee) has deemed on the screening form that IRB review is not needed, all projects must receive formal written clearance from the IRB Chair (or an IRB member designated by the Chair) **prior** to the start of data collection.

**Type of Project** (Check one)
☒ Departmental Research (faculty/student projects not externally funded and not indicated below)

☐ Graduate Thesis/Dissertation (IRB application should be submitted AFTER proposal defense)
**Advisor/Committee Chair** (& e-mail):

☐ Undergraduate Project (Senior thesis/capstone, research rookies, independent study)
**Advisor/Committee Chair** (& e-mail):

☐ Externally Sponsored Research
A complete copy of the grant proposal or contract must accompany this application form for IRB review to take place.

- Source of Funding:
- Title of grant proposal (if different from IRB protocol):
- Name of principal investigator on grant proposal:
- Office of Sponsored Projects file number (Note: this is not the grant number):
  OSP#

☐ Other
Specify:

**Part I. Purpose and Procedures:**

1) Describe the purpose of your study and the reason(s) this study is needed. Include any necessary background information and a description of your hypothesis or your research question.

According to the United States Department of Health and Human Services, adults over 65 years of age in the United States comprises 13.7% of the population or 43.1 million people. This is a 21% increase since 2002, and more than triple the older adult population since 1900. This population often suffers from at least one chronic condition or possibly multiple co-morbidities including gastrointestinal issues. A common condition is gastroesophageal reflux disease (GERD), affecting nearly 6 to 17% of the United States elderly population (Achem, 2014). A common prescription for GERD is a potent acid suppressant, such as proton pump inhibitors (PPIs). Proton pump inhibitors act by binding the hydrogen potassium ATPase pump of the parietal cells therefore, inhibiting gastric acid secretion from these cells in the stomach (Yang, 2010). This lack of gastric acid is thought to potentially have adverse effects on the absorption of vitamin B12 bound to food sources due to the requirement of adequate gastric acid for absorption. Malabsorption is an important condition to address to prevent deficiencies in an already vulnerable population. Vitamin B12 is vital for neurological function and a deficiency may contribute to cognitive decline that is often seen in the eldelry population. This investigation is very important in order to prevent the neurological symptoms that may be irreversible, yet a simple vitamin B12 supplement may help deter cognitive decline if related to a vitamin B12 deficiency.

Vitamin B12 status can be evaluated using various biomarkers. Current research has often utilized serum vitamin B12, but this marker is not a sensitive indicator of acute tissue status and often allows vitamin B12 defciciences to be undiagnosed. Methylmalonic acid (MMA) is an early indicator of vitamin B12 tissue depletion and has a higher sensitivity and specificity than serum vitamin B12. In the presence of a functional vitamin B12 deficiency, an accumulation of D-methylmalonyl-CoA will occur and this is then converted to MMA within the serum or urine. Urinary MMA (uMMA) is preferred over serum MMA (sMMA) because urinary methylmalonic acid (uMMA) is less likely to be falsely elevated due to renal insufficiency (Hill 2013). The non-invasive nature of provision of a urine sample versus a blood sample is also a benefit to utilizing uMMA.

The current body of research regarding B12 absorption and PPI use in the elderly have shown conflicting results. A study analyzing uMMA in the younger population using PPIs did not find any significant differences in uMMA values (Lukaszuk, 2013). In regards to the older population, studies have found that the elderly population using PPIs do show a decreased level of vitamin B12 compared to non-users (Rozgony,2010; Dharmarajan, 2008;Valuck,2004) while other studies have shown no effect of the use of PPIs on vitamin B12 status in the elderly (Hirschowitz, 2008; Den Elzen, 2008). Most of the research used serum vitamin B12 to indicate deficiency and this biomarker may miss a significant proportion of the population that may be deficient or have suboptimal B12 status.

2) The following items will help the IRB reviewers understand the step-by-step procedures of your study:
2A) Explain the participant **eligibility** and **exclusion** criteria that will be used.

Individuals will need to be between the ages of 55-90 years old. The exclusion criteria include history of Crohn's disease, ulcerative colitis, pernicious anemia, liver or kidney disease, adherence to a vegetarian diet or use of intramuscular shots of vitamin B-12 or nasal Nascobal. Diabetic individuals will not be exluded unless there is use of metformin. Individuals that have an implanted device such as a pacemaker are also excluded due to use of a bioelectrical impedance scale. If this criteria is met, participants will be asked to attend a 30 minute appointment at NIU in Wirtz 308A to complete a survey, have anthropometric measurements assessed and provide a urine sample. Participants will be asked to keep a three day food record prior to their appointment and submit at the time of their appointment.

2B) Explain the **recruitment** procedures (how will participants learn about the study?). If using the snowballing technique, please explain who contacts potential participants (other participants or the researcher). *Please attach recruitment scripts, flyers, or postings [Appendix A].*

Thirty men and women between the ages of 55-90 years old will be recruited throughout DeKalb, Illinois and within a 50 mile radius of DeKalb. Recruitment means will consist of advertisement at local assisted and independent living facilities and other community locations. An advertisement will be placed in the local newspaper. Flyers will also be posted throughout DeKalb, Illinois and within a 50 mile radius of DeKalb with study details and contact information. Social media will also be utilized to recruit individuals by posting the flyer on sites such as Facebook. The PPI group will be recruited first and the non-PPI group will then be recruited, matching for age and gender of the the PPI participants. See Appendix A for recruitment materials.

2C) Explain the **consent process** (verbal and/or written procedures for informing participants of the nature of the study and what they will do).
[*Please attach all documents (assent, consent, parent permission – **Appendix B**) that are appropriate for each group of subjects participating in the study. Consent forms should be prepared for adult participants (age 18 or over). Assent forms should be prepared for minor subjects appropriate to their ages, and permission form(s) for parents or legally authorized representatives should also be prepared. For children too young to comprehend a simple explanation of participation, parental permission is sufficient only if the research will provide direct benefit to the subject, a member of the subject's family, or other children with the same condition as the subject.*]

All participants will be informed of the risks and benefits of participation both verbally and in written form. There is no risk involved in this study and participants will be able to ask any outstanding questions before signing the consent form. See Appendix B for the written consent form.

2D) Describe the **data collection** procedures including what data will be collected, how it will be collected (include a description of any interventions to be used), the duration of participation in the study session(s), and how the session(s) will end.

Participants will report to Wirtz 308A at NIU for their appointment to measure anthropometrics and complete a short survey. Anthropometric data will be collected in order to determine equivalency between the PPI users and non-PPI users. Collection of this data will allow the reserachers to identify and consider any differences between the two groups regarding body composition
. Informed consent will be obtained before the appointment begins. Height will be measured using a wall mounted stadiometer. Weight, body fat percent, fat free mass, and body mass index (BMI) will be assessed using bioelectrical impedence (Biospace 520 or Tanita Body Composition Analyzer TBF-300A) with participants wearing light weight clothing and bare feet. A spot urine sample will be taken from participants for urinalysis to determine uMMA. Participants will use the restroom on the third floor of Wirtz Hall and will be provided a styrofoam cup marked with their identification number. Dr. Lukaszuk and Tashia Warner will then transfer at least 1 mL of this urine sample into a vial provided by Norman Labs in Ohio using a bulb pipette. These vials will contain 5 mg thymol as a preservative in order for samples to be mailed unrefrigerated These samples will be sealed, labeled with the participant number and stored at -20 degrees C at the Clinical Lab Sciences department on campus until all 30 participants have provided a urine sample. Safety precautions will be followed with the researchers wearing protective gloves when handling urine samples and disposing of excess urine appropriately.

A short survey (Appendix C) will also be administered and this will provide information regarding demographics, lifestyle behaviors, use of vitamin and mineral supplements and use of PPIs. Habits that will be questioned include smoking habits, use of vitamin B12, Vitamin B6 and/or folic acid supplementation, and use of specific PPIs. Participants will be provided with a three day food record (Appendix C) prior to their appointment and this completed log will be submitted at the time of their appointment.

There will be no intervention with the participants, although they will receive their anthropometric and urinalysis results for no cost and will have the opportunity to address a low vitamin B12 level, if applicable. The entire data collection process is estimated to take approximatley 30 minutes per participant.

Please note: It is the researcher's responsibility to seek out permission to use copyrighted materials. Please indicate whether you have permission to use any copyrighted materials for your project:

- ☐ Yes, I have permission to use any copyrighted materials for this project
- ☐ No, I do not yet have permission to use any copyrighted materials for this project
- ☒ This is not relevant for the materials being used in this project

2E) If applicable, explain the procedures for providing **compensation**.

| |
|---|
| n/a |

2F) If applicable, explain the procedures for **debriefing** participants. *Please attach a debriefing script or sheet [Appendix D]*

| |
|---|
| n/a |

**Reminder**: As appendices to this application, attach copies of all: **A) Recruitment** information [script/flyer/etc.], **B) Informed consent documents** [assent/parent permission/scripts/etc.], **C) Materials** [questionnaires/surveys/interview questions/listing of all information/data to be collected/etc.], **D) Debriefing information** [documents/scripts], **E) Referral** list [if appropriate]. It is the responsibility of the researcher to obtain any relevant permission for copyrighted materials. If the research involves an oral interview or focus group discussion that could evolve as it progresses, include a list of discussion topics and any "starter" questions for each topic that can reasonably be expected to be covered. If a *draft* of a written questionnaire or survey is attached, it should be clearly labeled as such and a final version must be submitted *before* data collection begins. **PLEASE NOTE THAT ANY ITEMS CAN BE ATTACHED AS SEPARATE DOCUMENTS IF NEEDED.**

**Part II: Research Participants**

3) Participant demographics:

- Gender: M ☐ F ☐ Both ☒
- Estimated age(s):

| |
|---|
| 55-90 years old |

- Are any subjects under age 18? Yes ☐ No ☒
- **Potentially** vulnerable populations (please indicate if any of the following groups are the **target population of the study**)
  - ☐ Pregnant women & fetuses
  - ☐ Prisoners
  - ☒ Decisionally impaired/mentally disabled
  - ☐ Specific ethnic group(s) (list in box):

| |
|---|
| |

If any potentially "vulnerable populations" have been indicated above, please explain the necessity for using this particular group, or if specific groups are excluded from the study, please indicate the exclusion criteria used.

| |
|---|
| Individuals who are decisionally impaired due to cognitive decline of age will be excluded from the study. |

- Target number of participants in the **entire study** (including controls) from start to finish (keep in mind that this is just an **estimate of the total**):

| |
|---|
| 30 |

4) Please explain any outside institutional (i.e., schools, hospitals) approval you will need to obtain and how approval will be sought. Provide scripts, letters, or emails providing any information that will be used to obtain needed approvals/permission. It is the responsibility of the researcher to follow all applicable policies of any outside institution(s).

| |
|---|
| n/a |

**Part III: Risk/Benefit assessment**

5) What knowledge/benefit(s) to the field will be gained from the study?

| |
|---|
| PPIs are currently obtained as over-the-counter or prescription drugs and are subject to potential abuse by those who need them. If the study shows a negative association between usage of PPI's and vitamin B12 levels, there will be a need for further studies and vitamin B12 education and warning PPI users to help prevent vitamin B12 deficiency. |

6) What direct benefit(s) are there to the participant(s) (if any) from the proposed research? *[For example, learning a new skill, psychological insight, teaching experience] [Please note that compensation is* ***NOT*** *considered a direct benefit.]*

All participants will be informed of their vitamin B12 status. Those with inadequate vitamin B12 levels will be advised to contact their healthcare providers.

7) Describe any potential risks (breach of confidentiality, economic, ethical, legal, physical, political, psychological/emotional, social, or other) to the subjects posed by the proposed research. (Note: Some studies may have "no reasonably foreseeable risks.") Investigators are required to report all unexpected and/or adverse events to the IRB. Therefore, it is important that you list all reasonably anticipated risks because unanticipated adverse events may need to be reported by NIU to OHRP.

There are no anticipated risks to the participants in this study. Confidential information breach is the only foreseeable risk of participation in this study.

8) Federal regulations require that researchers use procedures that minimize any risks to participants. What procedures will be used to minimize each risk and/or deal with the challenge(s) stated in "7" above?

All information will be locked away in a filing cabinet in the lead researcher's office and participants will be identified with a number to ensure no possible way to link the information to the participant. Any electronic documents with participant information will be encrypted.

9) If support services are required to minimize risk of harm to participants, explain what will be provided (list of services available – **Appendix E**). [*A resource list for the DeKalb area is available on the ORC website – if using this, please provide a copy with your application.]*

Since bioelectrical impedance will be used to assess body composition, potential participants with implants such as pacemakers are prohibited from the study

10) How do the potential benefits of the study *justify* the potential risks to the participants?

There is no foreseeable risk in this study.

**Part IV: Consent Document Variations**

11) Will audio, video, or film recording be used? Yes ☐ No ☒

If yes, specify the recording format to be used.

n/a

Please keep in mind that specific consent must be sought in the informed consent document(s) by including a **separate signature/date line giving consent** for recording. This is in addition to the signature/date line giving consent to participate in the research project.

12) Will this project require the use of consent/assent documents written in a language other than English? Yes ☐ No ☒

**Reminder**: If non-English documents will be used, please have the document translator **provide documentation** (email or written) that the translation is equivalent to the English version. [*This can be done after the protocol is approved in order to minimize the number of changes needed.]*

13) Are you requesting a **waiver of a signed** informed consent document? Yes ☒ No ☐

Please indicate the justification for requesting this waiver:

- ☒ The only record linking the subject to the research would be the signed consent document and the principal risk of the research would be breach of confidentiality.
- ☐ The research involves minimal risk to the subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., online surveys).

14) Are you requesting a **waiver/alteration** of some other aspect of the informed consent document? *[This section is relevant for studies involving **deception.**]* Yes ☐ No ☒

14a) Please explain which aspects of informed consent will be missing or altered along with a justification for the change.

n/a

14b) Please explain how the project meets all of the following criteria:

1) The research presents no more than minimal risk of harm to the participants.

n/a

2) The waiver/alteration will not adversely affect the rights or welfare of the participants.

n/a

3) The research could not practicably be carried out without the waiver or alteration.

n/a

4) Whenever appropriate, the participants will be provided with additional pertinent information after participation.

n/a

15) Will any HIPAA protected health information be collected as part of the data? Yes ☐ No ☒
If yes, describe the procedures for protecting the information.

n/a

*[Please provide a copy of your HIPAA disclosure form to be given to participants.]*

16) Will any protected school records be collected as part of the data? Yes ☐ No ☒
If yes, describe the procedures for protecting the information.

n/a

**Part V: Confidentiality and Anonymity**

17) Will identifying information be connected to the data (even through an identification key linking identities to a pseudonym or code that is kept separate from the data)? Yes ☒ (**confidential** data) No ☒ (**anonymous** data)

18) If you answered **yes to the above** question, describe precautions to insure the privacy of the subjects, and the confidentiality of the data, both in your possession and in reports and publications.

Participants will be assigned numbers as identification throughout the study. A master key will be kept on file indicating the participant name and their identification number. This key will be stored in the lead researcher's office in a locked filing cabinet separate from the rest of the data. Participant information and data will be stored in a separate locked filing cabinet from the master key or in an encrypted electronic document. Three years following publication of the study, the master key and raw data records will be destroyed by shredding paper documents and erasing computer files.

19) How will the records (data, recordings, and consent forms) be stored? **Also** indicate how long records will be kept and how and when they will be disposed of.
*[Note: Signed informed consent documents must be maintained for 3 years following completion of the study.]*

All data will be stored in a locked filing cabinet in the main investigator's office. Any electronic documents will be encrypted. Raw data will be shredded and electronic documents will be erased three years following the final publication of the data.

**Part VI: Does this project involving deception** Yes ☐ No ☒
***[complete this section only if your study includes deception]***

20) Describe the deception being used. Be sure to clarify whether this is deception by **omission** (an important aspect of the study is withheld from the participants) or **commission** (the participant is misled about some aspect of the study) or both. *[Complete item 14 if aspects of consent are missing.]*

n/a

21) Why is deception a necessary and unavoidable component of the experimental design?

n/a

22) Debriefing of participants will be:

☒ Immediate (directly following the research session)
☐ Delayed

☐ Full (all aspects of deception will be revealed)
☐ Partial (some aspects of deception will remain unexplained)

a) If debriefing is delayed, why is the delay necessary, and when will it occur?

n/a

b) If debriefing is partial, why is the partial debriefing necessary? Would the participant be harmed in any way by full debriefing?

n/a

c) If debriefing is partial, will full debriefing occur later?

n/a

d) Does the presence of deception increase risk of harm to the participants?

n/a

e) Is the respondent free to withdraw his/her data after being fully debriefed?

n/a

23) Who will provide the debriefing?

n/a

**Reminder**: Please include a copy of your debriefing script/sheet with this application **[Appendix D]**.

**Part VII: Credit and Compensation**

24) If participants will receive course credit for participation, please describe it below.

n/a

25) If participants will receive some other form of compensation for participation, please describe it below.

Participants will receive the results of their urinalysis and uMMA will indicate their vitamin B12 status. This allows particpants with low vitamin B12 status to address this with their healthcare provider.

26) Describe any alternative tasks that will be available for participants to earn the credit or compensation.

n/a

**Part VIII: Conflict of interest**

27) Do any of the researchers conducting this study have any potential conflicts of interest?
*[Conflicts of interest may include financial or personal interest, or any condition in which the investigator's judgment regarding a primary interest may be biased by a secondary interest.]* Yes ☐ No ☒

28) If yes to the above question, please describe the nature of the conflict of interest.

n/a

Please use the following link to access the NIU research conflict of interest policy:
http://www.research.niu.edu/divresearch/integrity/Research%20COI%20Policy%208-24-2012.pdf

**Part IX: Researcher Qualifications**

29) In addition to listing the investigators' names, indicate their qualifications to conduct procedures to be used in this study (specifically describe past experience conducting research with humans or how training will occur).

Dr. Lukaszuk and Tashia Warner will have direct contact with participants in the study. Dr. Judith Lukaszuk, professor and researcher, and Tashia Warner, graduate student, will be handling all of the urine samples and transferring the urine samples from styrofoam cups into vials provided by Norman Lab using a bulb pipette. Protective gloves will be utilized when handling urine samples. Tashia Warner will be verifying the three day food record information using diet analysis software and taking anthropometric measurements from participants using the bioelectrical impedance scale. Tashia Warner has completed the research methods course (FCNS 604) and will be supervised by Dr. Lukaszuk.

Dr. Lukaszuk has a Ph.D. in Exercise Physiology, a MS in Nutrition and Dietetics, and is a registered and licensed dietitian. Dr. Judith Lukaszuk has experience obtaining muscle samples from subjects and maintaining a sterile field while doing so. She also has experience obtaining blood samples and using gloves and disposing of unused blood samples using the biohazard container. Dr. Lukaszuk gained this experience in 1997-1999 while conducting her doctoral study on the Effect of creatine monohydrate on anaerobic exercise performance. The study was approved by the IRB and the University of Pittsburgh and the General Clinical Research Center at the University of Pittsburgh Medical Center as this study was funded by the NIH. Dr.Lukaszuk has more recent experience taking blood samples from subjects using a finger stick and obtaining urine samples from subjects from 2010-2012.

Tashia Warner has a BS in Nutrition, Dietetics and Hospitality Administration and will be working under the supervision of Dr. Lukaszuk. Tashia Warner also has experience obtaining anthropometric measurements utilizing the bioelectrical impedence scale last year working as a graduaste assistant for FCNS 415 and FCNS 416. Tashia Warner completed the CITI training in Fall 2013.

30) State the date of completion of **CITI Human Subjects Protection** training program(s) for the individuals listed in the above question. [*Note: NIU Policy requires that research investigators must complete appropriate training before conducting human subjects research.*] If you have comparable training, please attach certification indicating this. **CITI (Collaborative Institutional Training Initiative) training is thorough and well recognized: https://www.citiprogram.org/Default.asp?**

Dr. Lukaszuk attends the IRB inservice given at FCNS yearly.

Dr. Umoren attended a workshop on human subjects in August 2010 given by Sandra Arntz.

Dr. Shokrani has attended various related workshops and has years of experience working with clinical research.

Tashia Warner has completed the NIU online IRB training (CITI Human Subjects Protection Training Program) on November 9, 2013. She has also completed FCNS 604: Research Methods in Fall 2013 and ETR 521: Educational Statistics in Spring 2014.

**To be completed by investigator and confirmed by advisor (if student project) and departmental reviewer. Initials indicate all required parties ratify that application is complete:**

**Checklist of items required to accompany completed application form:**

1. _____ Complete grant proposal/contract (for externally funded projects)
2. _____ All surveys, questionnaires, interview questions, or other instruments to be used
3. _____ Subject recruitment/introductory materials
4. _____ Informed consent documents (must select at least one):

_____ Consent form for adults (if participants are age 18 or over)
_____ Assent form for minors (if participants are under age 18)
_____ Parental permission form (if participants are under age 18)

**Initial indicating all listed materials are attached and application is complete; INCOMPLETE APPLICATIONS WILL NOT BE PROCESSED. The investigator will be notified of deficiencies in the application via e-mail from the Office of Research Compliance (ORC); if no response is received by the ORC within five (5) working days the application will be considered void.**

Investigator _____ Advisor (if student project) _____ Department Chair/Designee _____

## REQUIRED SIGNATURES: ALL PROJECTS

CERTIFICATION

I certify that I have read and understand the policies and procedures for research projects that involve human subjects and that I intend to comply with Northern Illinois University Policy. Any changes in the approved protocol will be submitted to the IRB for written approval prior to those changes being put into practice unless it involves an immediate safety issue for the subject during a procedure. (In such instances, the researcher is required to promptly notify the IRB after the fact.) I also understand that all non-exempt projects require review at least annually.

_____

Investigator(s) Signature(s) Date

_____

Signature of Faculty Advisor (Student Project Only) Date

**Authorized Departmental Review:**

☐ Project qualifies for Administrative Review.
Cite the appropriate exempt category: ☐

☐ Project qualifies for Subcommittee Review.
Cite the appropriate expedited category: ☐

☐ Project is referred for review by the convened IRB.

_____

Signature of Authorized Departmental Reviewer Printed name Date

Return this form, together with necessary documentation, to the Office of Research Compliance, Lowden Hall, 301. For information or additional assistance with the approval process, please call the office at (815) 753-8588 or access the ORC web page at www.orc.niu.edu.

NORTHERN ILLINOIS UNIVERSITY
Office of Research Compliance and Integrity
*Lowden Hall 301 · DeKalb, IL 60115-2584*
*815-753-8588 · Fax 815-753-1631 · www.niu.edu/orci*

# Approval Notice

**Initial Review**

06-Nov-2014

TO: Judith Lukaszuk
Family, Consumer and Nutrition Sciences

RE: Protocol # HS14-0344 "**Methylmalonic acid levels in older adult chronic proton pump inhibitor users**"

Your **Initial Review** submission was reviewed and approved under **Expedited** procedures by Institutional Review Board #2 on **06-Nov-2014**. Please note the following information about your approved research protocol:

Protocol Approval period: **06-Nov-2014 - 05-Nov-2015**

If your project will continue beyond that date, or if you intend to make modifications to the study, you will need additional approval and should contact the Office of Research Compliance and Integrity for assistance. Continuing review of the project, conducted at least annually, will be necessary until you no longer retain any identifiers that could link the subjects to the data collected. Please remember to use your **protocol number** (**HS14-0344**) on any documents or correspondence with the IRB concerning your research protocol.

**Please note that the IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.**

Unless you have been approved for a waiver of the written signature of informed consent, this notice includes a date-stamped copy of the approved consent form for your use. NIU policy requires that informed consent documents given to subjects participating in non-exempt research bear the approval stamp of the NIU IRB. This stamped document is the only consent form that may be photocopied for distribution to study participants.

It is important for you to note that as a research investigator involved with human subjects, you are responsible for ensuring that this project has current IRB approval at all times, and for retaining the signed consent forms obtained from your subjects for a minimum of three years after the study is concluded. If consent for the study is being given by proxy (guardian, etc.), it is your responsibility to document the authority of that person to consent for the subject. Also, the committee recommends that you include an acknowledgment by the subject, or the subject's representative, that he or she has received a copy of the consent form. In addition, you are required to promptly report to the IRB any injuries or other unanticipated problems or risks to subjects and others. The IRB extends best wishes for success in your research endeavors.

# APPENDIX E

# IBC APPLICATION AND APPROVAL LETTER

*Northern Illinois University*

**Institutional Biosafety Committee**

**Application for Approval**

| **Leave Blank** |
|---|
| **ORC Protocol #** |
| **Approval Date:** |
| **Expiration date:** |

## *I. Project Identification*

***Project Title:*** **Methylmalonic Acid Levels in Older Adult Chronic PPI Users**

| *Investigator Name(s)* | *Department* | *Telephone* |
|---|---|---|
| Dr. Judith Lukaszuk, PhD, RD, LDN | FCNS | 815-753-6352 |
| Dr. Josephine Umoren, PhD | FCNS | 815-753-6351 |
| Dr. Masih Shokrani, PhD | AHCD | 815-753-6323 |
| Tashia Warner, Graduate Student | FCNS | 815-474-7339 |

| *Grant Proposal Titles:* | *OSP Project #* |
|---|---|
| n/a | n/a |

***Project Objective:***

To determine the effect of proton pump inhibitor use on vitamin $B_{12}$ levels in older adults 55-90 years old.

***Project Start and End Dates: Fall 2014 to Spring 2015***

This study will begin once IRB/IBC approval are granted.

***Research Location:***

NIU Campus: Data will be collected in the NIU Nutrition Lab, Room 308A Wirtz Hall

## *II. Project Description*

**For all research involving biohazards as defined on page A-2:**

Summarize the proposed activity. In particular, describe what systems you plan to start with, what your endpoint is, what types of manipulations you plan to use to achieve that goal, and whether you anticipate any complications in that process. Use an additional page, if necessary. Also, provide any additional material (publications, diagrams) that might help the IBC review the activity.

This experiment will consist of data collection in the nutrition lab (Wirtz 308A) at Northern Illinois University. Subjects will be recruited by posting flyers within the DeKalb community and within a 50 mile radius of DeKalb. If interested individuals meet the criteria of the study, they will be provided with a three day food record either via email, mail or in person and the participant will then be required to complete and bring the food record to their appointment. The three day food record will track participant intake for three consecutive days

Participants will be provided with a consent form to sign prior to collection of the three day food record and prior to participation in the study. Once the consent form is signed, subjects will be asked to complete a short survey regarding demographics, lifestyle habits and medication use. Participants will be given as

much time as needed to complete the survey. Anthropometrics will be taken utilizing a bioelectrical impedance scale (Biospace 520 or Tanita Body Composition Analyzer TBF-300A) and then the subject will be instructed to provide a urine sample using a sterile cup. The sterile cup will be labeled with the participant's name and study number and participants will use the bathroom next to Wirtz 308A. The participant will then transport the sterile cup back to Wirtz 308 or the researcher will collect the cup from the participant upon their exit from the bathroom. While wearing protective gloves, Tashia Warner and Dr. Lukaszuk will transfer at least 1 mL of the urine sample from the sterile cup using a bulb pipette to a vial provided by Norman Lab. The vials will be filled with 5 mg thymol as a preservative to allow for unrefrigerated shipping in order to be analyzed. All pre-labeled vials of urine will be transported to Clinical Lab Sciences to be frozen at -20°C until analyses. Once all subjects are recruited and 30 urine samples are collected, the urine vials will be shipped overnight to Norman Lab to assess for methylmalonic acid levels.

Exclusion criteria include history of Crohn's disease, ulcerative colitis, pernicious anemia, liver or kidney disease, use of metformin, adherence to a vegetarian or vegan diet or use of intramuscular shots of vitamin B12 or nasal Nascobal (Strativa Pharmaceuticals, Spring Valley, NY). All participants will be informed of the risks and benefits associated with this study and will be required to give written consent to participate in accordance with the Institutional Review Board at Northern Illinois University.

## *III. Personnel and Experience*

List the names, positions, and (very briefly) the years of experience in handling biohazard related materials of all investigative staff (students and technicians) currently working on or involved with this research protocol. Please use an additional page, if necessary. **You must update this information whenever your staff changes.**

| *Name* | *Position* | *Phone* | *Degree* | *Years Experience* |
|---|---|---|---|---|
| Dr. Joesphine Umoren | Associate Professor and Researcher | 815-753-6351 | PhD. | 20 |
| Dr. Judith M. Lukaszuk | Associate Professor and Researcher | 815-753-6353 | Ph.D. | 12 |
| Dr. Masih Shokrani | Assistant Professor and Researcher | 815-753-6323 | PhD | 18 |
| Tashia Warner | Graduate Student | 815-474-7339 | BS | <1 |

## Emergency Information

Provide the names and telephone numbers of at least two personnel who are qualified to handle biohazard-related emergencies in your laboratory, beginning with yourself and the person normally in charge when you are absent. **If both have the same home phone, please provide a third contact person.**

| *Qualified Emergency Contact* | *Work Telephone* | *Home Telephone* |
|---|---|---|
| Dr. Umoren | 815-753-6351 | |
| Dr. Lukaszuk | 815-753-6353 | 847-931-4876 |
| Dr. Masih Shokrani | 815-753-6323 | |

## *IV. Biohazard Summary*

For each type of biohazard used in this protocol, complete the following chart. **Attach an additional page if needed.**

| *Agent/ Organism(s)* | *Source* | *Purpose or Use* | *Biosafety Level* |
|---|---|---|---|
| Urine specimen | Subject will be asked to void | Evaluation of urinary methylmalonic acid | Level 2 |

## *Recombinant DNA*

**Complete this section for *all* activities involving recombinant DNA.** If you cannot locate the appropriate classification(s), a letter of classification from the relevant agency *must* be attached to this form. List host, vector, and sources so that *H1*, *V1*, and *S1* correspond.

*Note:* The IBC has pre-approved certain common hosts and vectors. The list is available from the Office of Research Compliance or the IBC chair. If your hosts and vectors do not appear on the list, you must provide the complete genotype of the host organism(s) and a diagram of the vector(s) to be used.

| *Host Organism with Strain Number* | | *Genotype* | *Biosafety Level* |
|---|---|---|---|
| H1 | N/A | | |
| H2 | N/A | | |
| H3 | N/A | | |
| H4 | N/A | | |

| *Corresponding Vectors* | |
|---|---|
| V1 | N/A |
| V2 | N/A |
| V3 | N/A |
| V4 | N/A |

| *Inserted DNA Sources* | |
|---|---|
| S1 | N/A |
| S2 | N/A |
| S3 | N/A |
| S4 | N/A |

| *Do you use a helper virus?* | *Do you deliberately attempt to obtain expression of a foreign gene? If yes, what protein is produced?* |
|---|---|
| No | No |

If your project involves large-scale (>10 liters of culture) research or production or uses transgenic plants, additional guidelines may apply. Contact the Office of Research Compliance for assistance.

## *V. Laboratory Safety Procedures and Training*

**Experience with Biohazards**

Describe your training and experience in handling biohazards safely.

Dr. Umoren has experience collecting and safely handling urine, blood and fecal samples from her work at the University of Nebraska.

Dr. Judith Lukaszuk has experience obtaining muscle samples from subjects and maintaining a sterile field while doing so. She also has experience obtaining blood samples and using gloves and disposing of unused blood samples using the biohazard container. Dr. Lukaszuk gained this experience in 1997-1999 while conducting her doctoral study on the effect of creatine monohydrate on anaerobic exercise performance. The study was approved by the IRB and the University of Pittsburgh and the General Clinical Research Center at the University of Pittsburgh Medical Center as this study was funded by the NIH.

Dr Shokrani is a certified clinical laboratory scientist and has extensive experience in handling human specimen such as urine and blood. Dr.Shokrani has published his work which was based on using and handling human specimen.

Tashia Warner is a graduate student and has her BS in Nutrition and Dietetics. She will be supervised by Dr. Lukaszuk during the data collection process.

**Staff Training Requirements and Procedures**

I certify that the following training and information is provided for all lab personnel:

- The biosafety containment level requirements are posted in the laboratory.
- All investigative staff are informed orally about the policies and procedures concerning handling and disposing of recombinant DNA molecules and organisms as required, based on the appropriate biosafety containment level.
- All investigative staff who have not taken microbiology and recombinant DNA courses are given appropriate instructions and must participate in laboratory demonstrations of the procedures for properly handling and disposing of recombinant DNA/organism-contaminated waste and laboratory materials.

**Laboratory Environment Safeguards**

I certify that the following laboratory environment safeguards are enforced at all times:

- Access to biohazard areas is restricted.
- Lab benches are disinfected before and after every experiment.
- Mouth pipetting is prohibited; mechanical pipetters are provided.

- Surgical gloves are required whenever lab personnel are working with biohazardous materials.
- Laminar flow cabinets are used according to the criteria specified by the CDC and the NIH in their publication *Biosafety in Microbiological and Biomedical Laboratories*
- Safety glasses used according to the criteria specified by the Occupational Safety and Health Administration (OSHA).
- Syringes, needles, broken glass, and all other sharps are disposed of according to the procedures prescribed by the CDC and the NIH in their publication *Biosafety in Microbiological and Biomedical Laboratories*

**Containment and Disposal Safeguards**

I certify that the appropriate containment and disposal safeguards are enforced at all times:

- Disposable materials that are contaminated with recombinant or infectious material are put into a biohazard bag and autoclaved.
- Non-disposable materials that are contaminated with recombinant or infectious materials are inactivated by autoclaving -OR- cleaning with bleach.

***Certifications***

I certify that the information provided in this protocol submission form is accurate; and any protocol changes, including the DNA being cloned, the vector, the host organism, or any other toxic or infectious agents, will be submitted to the IBC for approval prior to initiation.

I further certify that I have read and will comply with all relevant publications, including but not limited to the NIU *Institutional Biosafety Committee Policy*, the CDC/NIH *Biosafety in Microbiological and Biomedical* and the Department of Health and Human Services National Institutes of Health *Guidelines for Research Involving Recombinant DNA Molecules*.

***Signature(s) of Principal Investigator(s)*** ***Date***

The signature below certifies that the department chair understands the proposed protocol, finds it to be appropriate, and confirms that it can be conducted safely and securely in accord with federal guidelines and institutional policy.

***Signature of Department Chair*** ***Date***

NORTHERN ILLINOIS UNIVERSITY
Office of Research Compliance and Integrity
*Lowden Hall 301 - DeKalb, IL 60115-2584*
*815-753-8588 - Fax 815-753-1631 - www.niu.edu/orci*

November 19, 2014

MEMORANDUM

TO: Judith Lukaszuk
School of Family, consumer & Nutrition Sciences

RE: Approval of the use of recombinant DNA and/or pathogenic substances in research and/or instruction

The following project involving the use of recombinant DNA and/or pathogenic substances was reviewed and approved by the Institutional Biosafety Committee at its November 19th meeting:

| | |
|---|---|
| Title: | *Methylmalonic Acid Levels in Older Adult Chronic PPI Users* |
| IBC/ORC #: | S14-0012 |
| Biosafety Level: | BL-2 |
| Lab: | WZ 308A |

This approval is effective for one year from the date of review, until November 19, 2015. If your project will continue beyond that date, you should contact the Office of Research Compliance and Integrity (815-753-9251) for assistance with additional approval procedures.

# APPENDIX F

# CONSENT FORM

**Consent Form**

I do hereby consent to take part in the study regarding the effect of stomach acid lowering medications such as proton pump inhibitors on vitamin $B_{12}$ levels in older adults by Judith Lukaszuk, Josephine Umoren, Masih Shokrani, and Tashia Warner at Northern Illinois University. I have been informed that the purpose of the study is to compare vitamin $B_{12}$ levels among older adults who use these stomach acid lowering medications with those who do not use these medications by measuring the amount of urinary methylmalonic acid found in the urine as an indication of each person's vitamin $B_{12}$ levels.

I understand that as a participant of this study, I will complete a three day food record for three (3) consecutive days, a short survey that includes information about demographics, dietary habits, vitamin and mineral supplement intake and past medical history, have my height, weight and body composition measured, and provide a urine sample. I also understand that my body composition will be measured using bioelectric impedance analysis. I understand that collection of this data will take place in the nutrition laboratory in Wirtz Hall 308A at Northern Illinois University and will take approximately 30 minutes total.

I understand that my participation is voluntary and I may with draw from this study at any time without penalty or prejudice. I understand that if I wish to obtain further information regarding my rights as a research subject, I may contact the Office of Research Compliance at Northern Illinois University at (815) 753-8588.

I understand that all records are held in confidence.

I understand that the benefits of this study include determining my body composition and my vitamin $B_{12}$ status.

I understand that there is no risk to this study because there is no alteration in dietary or activity patterns requested.

I understand that any information obtained in connection with this study and that may identify me individually will be kept confidential at all times.

I understand that my consent to participate in this study does not constitute a waiver of any legal rights or redress I might have as a result of my participation. I acknowledge that I have received a copy of this consent form.

Date Signature of Participant

Date Signature of Investigator

APPROVED
NOV 06 2014
BY NIU I.R.B.
VOID ONE YEAR
FROM ABOVE DATE

APPENDIX G

THREE DAY FOOD RECORD

Adapted from Lukaszuk et al

# Three Day Food Record

Instructions:

1. Measure and record your food and beverage intake for three consecutive days. These can be any three days. It does not have to be three days prior to your appointment at NIU. Be sure to write down exactly what it is you ate, drank (including water, protein supplements and vitamin and mineral supplements), the amount and the brand name of the product. Use cups, teaspoons, tablespoons, ounces, slices or inches as your units of measurement.
2. Continue your normal eating patterns during this record to ensure an accurate representation of your normal diet.
3. Use two weekdays and one weekend day.

Adapted from Lukaszuk et al

| Food Log | | | |
|---|---|---|---|
| **Day 1** | **Date:** | | |
| **Supplements:** | | | |
| **Time** | **Meal** | **Portion** | **Food Item** |
| | | | |

Adapted from Lukaszuk et al

| Food Log | | | |
|---|---|---|---|
| **Day 2** | **Date:** | | |
| **Supplements:** | | | |
| **Time** | **Meal** | **Portion** | **Food Item** |
| | | | |

Adapted from Lukaszuk et al

| Food Log | | | |
|---|---|---|---|
| **Day 3** | **Date:** | | |
| **Supplements:** | | | |
| **Time** | **Meal** | **Portion** | **Food Item** |
| | | | |

# APPENDIX H

# SURVEY FOR PPI USERS

# Survey for PPI Users

Name: _____

Age: _____

Gender: _____

*Demographics*

Which of the following best describes you?

Caucasian: _____

African/African-American: _____

Asian/Asian-American: _____

Hispanic/Latino: _____

Native American: _____

Other: _____

*Lifestyle and Eating Habits*

Are you an ex-smoker? Circle One: Yes or No

Do you currently smoke? Circle One: Yes or No

If yes, how many cigarettes or cigars do you smoke per day? _____

_____

Which of the following diets do you currently follow?

Gluten Free: _____

Lactose Free: _____

No Restrictions: _____

Other: _____

How many meals per day do you eat? _____

How many snacks per day do you eat? _____

How would you best describe your appetite?

Excellent: _____

Good: _____

Fair: _____

Poor: _____

Do you eat red meat? Circle One: Yes or No

If yes, how many days per week do you eat red meat? _____

Do you eat/drink milk or milk products?

Circle One: Yes or No

If no, do you eat/drink soy, almond, rice or coconut milk substitute products?

Circle One: Yes or No

How many days per week do you eat/drink milk products or milk substitute products? _____

Which of the following best describes your exercise activity?

7 days/week: _____
6 days/week: _____
5 days/week: _____
4 days/week: _____
3 days/week: _____
2 days/week: _____
1 day/week: _____
None: _____

Which of the following best describes the intensity of your activity?

Sedentary (i.e. normal activities of daily living only): _____
Low (i.e. able to sing while exercising): _____
Moderate (i.e. conversation possible, but winded): _____
Vigorous (i.e. gasping for breath): _____

How long does your typical exercise session last?

Less than 20 minutes: _____
20-29 minutes: _____
30-39 minutes: _____
40-49 minutes: _____
50-59 minutes: _____
60 minutes or more: _____
Not Applicable: _____

*Medication/Supplement Use*

What acid suppressant medication are you currently taking? _____

_____

_____

What dosage? _____

Are you currently taking any other medications?

Circle One: Yes or No

If yes, please list. _____

_____

_____

_____

Are you currently taking an oral vitamin and/or mineral supplement? If yes, please list supplement(s).

_____

_____

_____

Are you currently taking an oral supplemental form of vitamin $B_{12}$?

Circle One: Yes or No

If yes, what dosage are you currently taking? _____

If yes, how long have you been taking this supplement? _____

Are you currently taking an oral supplemental form of vitamin $B_6$?

Circle One: Yes or No

If yes, what dosage are you currently taking? _____

If yes, how long have you been taking this supplement? _____

Are you currently taking an oral supplemental form of folic acid?

Circle One: Yes or No

If yes, what dosage are you currently taking? _____

If yes, how long have you been taking this supplement? _____

# APPENDIX I

# SURVEY FOR NON-PPI USERS

# Survey for Non-PPI Users

Name: _____

Age: _____

Gender: _____

*Demographics*

Which of the following best describes you?

Caucasian: _____

African/African-American: _____

Asian/Asian-American: _____

Hispanic/Latino: _____

Native American: _____

Other: _____

*Lifestyle and Eating Habits*

Are you an ex-smoker? Circle One: Yes or No

Do you currently smoke? Circle One: Yes or No

If yes, how many cigarettes or cigars do you smoke per day? _____

_____

Which of the following diets do you currently follow?

Gluten Free: _____

Lactose Free: _____

No Restrictions: _____

Other: _____

How many meals per day do you eat? _____

How many snacks per day do you eat? _____

How would you best describe your appetite?

Excellent: _____

Good: _____

Fair: _____

Poor: _____

Do you eat red meat? Circle One: Yes or No

If yes, how many days per week do you eat red meat? _____

Do you eat/drink milk or milk products?

Circle One: Yes or No

If no, do you eat/drink soy, almond, rice or coconut milk substitute products?

Circle One: Yes or No

How many days per week do you eat/drink milk products or milk substitute products? _____

Which of the following best describes your exercise activity?

7 days/week: _____
6 days/week: _____
5 days/week: _____
4 days/week: _____
3 days/week: _____
2 days/week: _____
1 day/week: _____
None: _____

Which of the following best describes the intensity of your activity?

Sedentary (i.e. normal activities of daily living only): _____
Low (i.e. able to sing while exercising): _____
Moderate (i.e. conversation possible, but winded): _____
Vigorous (i.e. gasping for breath): _____

How long does your typical exercise session last?

Less than 20 minutes: _____
20-29 minutes: _____
30-39 minutes: _____
40-49 minutes: _____
50-59 minutes: _____
60 minutes or more: _____
Not Applicable: _____

*Medication/Supplement Use*

Are you using any antacid medications that are NOT PPIs?

Circle One: Yes or No

If yes, what type? _____

Are you currently taking any other medications?

Circle One: Yes or No

If yes, please list. _____

_____

_____

_____

Are you currently taking an oral vitamin and/or mineral supplement? If yes, please list supplement(s).

_____

_____

_____

Are you currently taking an oral supplemental form of vitamin $B_{12}$?

Circle One: Yes or No

If yes, what dosage are you currently taking? _____

If yes, how long have you been taking this supplement? _____

Are you currently taking an oral supplemental form of vitamin $B_6$?

Circle One: Yes or No

If yes, what dosage are you currently taking? _____

If yes, how long have you been taking this supplement? _____

Are you currently taking an oral supplemental form of folic acid?

Circle One: Yes or No

If yes, what dosage are you currently taking? _____

If yes, how long have you been taking this supplement? _____