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A Comparison of Nebulized Vitamin B12 Versus Oral Vitamin B12 Supplementation

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ABSTRACT

Vitamin B12 is important for various processes in the human body including DNA synthesis, red blood cell production, energy production, fatty acid synthesis, and for the conversion of homocysteine into methionine. Vitamin B12 deficiency is becoming more prevalent. The purpose of this study was to evaluate if a different route of administration, i.e. nebulization, was a viable option for increasing serum levels of vitamin B12 in both exercisers and non-exercisers. The increasing number of people that are vitamin B12 deficient is due to several factors including medication use, elective gastric bypass surgery, and an increasing elderly population. There are currently two common ways to treat an individual who is deficient in vitamin B12; daily oral supplementation or intramuscular injection of vitamin B12. For this study, participants were placed in one of four groups (non-exercise oral B12, non-exercise nebulized B12, exercise oral B12, and exercise nebulized B12) and received either 28 consecutive days of oral B12 supplementation or once-weekly nebulized B12 treatments for four consecutive weeks. Repeated measures ANOVA indicated there was no significant time by group interaction ($F[1,33] = 2.352$, $p = .135$) or time by exercise interaction ($F[1,33] = 3.013$, $p = .087$). Also, there were no significant differences for the time by group by exercise interaction ($F[1,33] = 1.472$, $p = .234$). Although statistical significance was not achieved, nebulizing vitamin B12 did increase serum B12 levels more than oral supplementation, and is a viable option for increasing serum B12 levels. Investigating other populations such as the elderly, vegetarians, or individuals taking medications that impair with the absorption of vitamin B12 may be beneficial to determine if similar increases in serum B12 levels is expected in such populations.

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CHAPTER ONE

INTRODUCTION

People receive their energy for daily activities from foods and liquids they consume. According to the United States Department of Agriculture and the United States Department of Health and Human Services Dietary Guidelines for Americans 2010, estimates of caloric intake range from 1,600 to 2,400 calories per day for adult women and 2,000 to 3,000 calories per day for adult men, depending on age and physical activity level (United States Department of Agriculture, 2010). Within these foods are vitamins and minerals necessary for normal human function, development, and physiology. Vitamins are organic substances made by plants or animals. Minerals are inorganic elements that come from the earth; soil and water and are absorbed by plants. Animals and humans absorb minerals from the plants they eat. Vitamins and minerals are nutrients that your body needs to grow and develop normally (Centers for Disease Control, 2012). Vitamins are subdivided into two classifications: fat soluble and water-soluble. The fat-soluble vitamins include vitamins A, D, E, and K. Every other vitamin is water-soluble. One water-soluble vitamin of particular interest is vitamin B12, also known as cobalamin.

Vitamin B12 is a water-soluble vitamin that is necessary in the human body for DNA synthesis, red blood cell production, energy production, fatty acid synthesis, nervous system maintenance (Butler et al., 2006), as well as a regulatory vitamin for the conversion of homocysteine into methionine. Homocysteine is an amino acid found in the blood that can be converted into methionine with the help of folate or converted into cysteine. An elevated homocysteine level is an independent risk factor for cardiovascular disease. Methionine is an

essential amino acid and it is a intermediate step in the synthesis of cysteine, carnitine, taurine, lecithin, and phosphatidylcholine. Vitamin B12 can be found in such foods as beef liver, fish, meat, poultry, eggs, milk, and other dairy products. Some breakfast cereals and nutritional yeasts contain vitamin B12 as well as other food products that may be fortified with vitamin B12. The recommended intake for vitamin B12 for normal adults is 2.4 micrograms per day (Goldman & Ausiello, 2004).

The typical absorption of vitamin B12 is dependent upon several steps. Upon consumption, vitamin B12 binds to the glycoprotein haptocorrin in the stomach. This glycoprotein serves to protect the vitamin B12 from the acidic environment of the stomach. Once vitamin B12 passes into the duodenum, the presence of gastric and pancreatic juices release the B12 from haptocorrin and B12 is immediately bound to intrinsic factor. Intrinsic factor is a glycoprotein made by the parietal cells of the stomach. Once the vitamin B12 is bound to intrinsic factor it travels through the small intestine to the ilium where ileal mucosal cells uptake the intrinsic factor bound vitamin B12 (Hillman, 1996). Once the B12 is absorbed it unbinds with intrinsic factor and binds to transcobalamin II. This form now exits the cells of the ilium into the blood where it is transported to the liver where it can be processed and utilized by the body for the previously mentioned processes of DNA synthesis, red blood cell production, energy production, fatty acid synthesis and the conversion of homocysteine into methionine. The vitamin B12 bound to transcobalamin II is rapidly cleared from plasma and distributed to parenchymal cells (hepatocytes) where it can be stored. The liver is the preferred storage facility for vitamin B12 (Hillman, 1996), and the liver can store enough vitamin B12 to prevent deficiency for at least 1 year and maybe several years (Guyton & Hall, 2000).

Vitamin B12 deficiency was first described in 1849, and was considered to have a fatal outcome until 1926 when a diet of liver, high in vitamin B12, was shown to slow the disease process (O'Leary & Samman, 2010). Today, vitamin B12 deficiency is becoming a more prevalent problem in our society. In the United States, more than 20% of the elderly have serum vitamin B12 concentrations that indicate depletion, and an additional 6% have deficiency (L. Allen, Rosenberg, Oakley, & Omenn, 2010). It was also found that prevalence of vitamin B12 deficiency in younger adults may be higher than previously thought (Tucker et al., 2000). The evidence from the Framingham Offspring Study indicated that percentages of the populations in three age groups (26-49 years, 50-64 years, and 65 years and older) with deficient blood levels (<148 pmol/L or 200 pg/mL) of vitamin B12 was similar being 8.3%, 8.8% and 8.7% respectively (Tucker et al., 2000).

Cecil's Textbook of Medicine (2004) indicates two causes for vitamin B12 deficiency. They are decreased ingestion and impaired absorption. Decreased ingestion is due to poor diet, lack of animal products and strict vegetarianism. There are etiologic mechanisms for impaired absorption related vitamin B12 deficiency. First is failure to release cobalamin from food protein. This happens as a natural part of aging as well as with partial gastrectomies. Second is intrinsic factor deficiency. A lack of intrinsic factor can result from total gastrectomy surgeries, destruction of the gastric mucosa by caustics (a corrosive agent) and a congenital abnormal or absence of the intrinsic factor molecule. These intrinsic factor deficiencies lead to pernicious anemia. The third etiologic mechanism for impaired vitamin B12 deficiency is chronic pancreatic disease. When the pancreas is diseased it may not produce adequate buffering agents to allow vitamin B12 to release from haptocorrin and to subsequently bind to intrinsic factor. The fourth etiology is competitive parasites. These would include bacteria in the diverticula of

the bowel and or fish tapeworm infestations (diphyllobothrium latum). The fifth etiology for impaired absorption of vitamin B12 is intrinsic intestinal diseases. This would include ileal resection, Chron's disease, radiation ileitis, tropical sprue, celiac disease, lymphoma, scleroderma, drug induced malabsorption and congenital selective malabsorption, and Imerslund-Grasbeck syndrome (Goldman & Ausiello, 2004).

According to the National Institutes of Health Vitamin B12 fact sheet, symptoms of vitamin B12 deficiency can include megaloblastic anemia, fatigue, weakness, constipation, loss of appetite, weight loss, numbness and tingling of hands and feet, difficulty maintaining balance, depression, confusion, dementia, poor memory and soreness of the mouth or tongue (National Institutes of Health, 2012). Vitamin B12 deficiency is currently treated by three different methods: oral supplementation, intramuscular injection, and less commonly, prescription nasal gel. The National Center on Birth Defects and Developmental Disabilities and the Centers for Disease Control have note that there are varying recommendations for treatment of vitamin B12 deficiency including:

1. 1 mg of B12 injected once per week for 8 weeks then once per month for life
2. Daily 1 mg injections for 1-2 weeks then maintenance dose of 1mg every 1 to 3 months (Oh & Brown, 2003)
3. Oral supplementation is typically 1 mg per day (Evatt, Mersereau, Bobo, Kimmons, & Williams, 2010).

The Institute of Medicine (IOM) did not establish an upper limit for vitamin B12 because of its low potential for toxicity. The IOM states that no adverse effects have been associated with excess vitamin B12 intake from food and supplements in healthy individuals (National Institutes of Health, 2012). Vitamin B12 is considered safe, even at levels much higher than the

recommended dose. It has not been shown to be toxic or cause cancer, birth defects, or mutations and treatment is safe, effective, and has no known toxicity level (Evatt et al., 2010).

PURPOSE OF THE STUDY

Vitamin B12 is important for various processes in the human body including DNA synthesis, red blood cell production, energy production, fatty acid synthesis, and vitamin B12 serves a regulatory vitamin for the conversion of homocysteine into methionine. Vitamin B12 deficiency is becoming more prevalent. The purpose of this study was to evaluate if a different route of administration, i.e. nebulization, is a viable option for increasing serum levels of vitamin B12 in both exercisers and non-exercisers.

NULL HYPOTHESES

$H_0$1 = There will be no statistical interaction between administration (oral vs. nebulized) and exercise group (exercise vs. non-exercise).

RESEARCH HYPOTHESES

H1 = Four nebulized vitamin B12 treatments (one treatment per week for four consecutive weeks) will increase serum B12 levels in both the non-exercise and exercise group.

H2 = Twenty-eight consecutive days of oral vitamin B12 supplementation will increase serum B12 levels in both the non-exercise and exercise group.

LIMITATIONS

1) Results of this study can only be generalized to a healthy population aged 18-49 years.
2) All participants are volunteers.
3) All participants self-reported on physical activity level.

4) All participants self-reported on taking the oral supplements every day for the duration of the study

DEFINITION OF TERMS

1) Nebulize – to reduce to a fine spray.
2) Malabsorption – faulty absorption of nutritive material.
3) Cobalamin – a water-soluble vitamin needed for normal brain function, nervous system function, and formation of blood. It is also affects DNA synthesis, fatty acid synthesis, and energy production.
4) Haptocorrin – a protein in humans, which binds to vitamin B12 to protect vitamin B12 from the acidic environment of the stomach.
5) Intrinsic factor – a glycoprotein produced in the parietal cells of the stomach, which binds to vitamin B12. Intrinsic factor bound vitamin B12 is necessary for absorption of vitamin B12 at the ileum.
6) Transcobalamin II – a carrier protein that binds to vitamin B12 in the ileal cells to transport vitamin B12 from the ileum, into portal circulation to be processed in the liver.
7) Fit but not trained: participants who exercise at a level of at least 65% of their age predicted maximum heart rate on at least 3 days per week
8) Exercise group-- the participants who self-report that they exercise and are fit but not trained
9) Non-exercise group -- the participants who self-report that they do not exercise at a level of at least 65% of their age predicted maximum heart rate on at least 3 days per week

SIGNIFICANCE OF THE STUDY

A review of literature revealed that there are an increasing number of people that are vitamin B12 deficient. This increase is due to several factors. More people are taking prescription medications including proton pump inhibitors and metformin for conditions such as gastroesophageal reflux and diabetes mellitus. Also, people are electing to have surgeries such as gastric bypass, which will reduce absorption of vitamin B12. There is an increasing population of older individuals. With this aging population comes age-related physiologic changes such as atrophic gastritis which may reduce normal absorption of vitamin B12. Vitamin B12 is important and once it has been determined that an individual is deficient in vitamin B12 the course of therapy is vitamin B12 supplementation for life. There are currently two common ways to treat an individual who is deficient in vitamin B12; daily oral supplementation or intramuscular injection of vitamin B12. The medical and pharmaceutical industries have identified medication adherence as a health-care problem and are currently investigating ways to increase an individual's adherence to their required medication(s). Literature suggests that approximately half of people abandon their treatments.

Oral supplementation of vitamin B12 for deficiency must rely on persons adhering to their daily course of treatment for the duration of their lifetime. Conversely, intramuscular injections of vitamin B12 would mean initial weekly visits to the individual's health care provider. Eventually the frequency of visits would reduce, to monthly visits to their provider, but these monthly visits would be necessary for the duration of the individual's lifetime. Because of the increasing number of persons becoming vitamin B12 deficient, coupled with the confounding factors of cost, health, and age related physiologic changes that may cause someone to become deficient in vitamin B12, it is important to investigate all possible routes of

administration for vitamin B12. There have been many studies that show oral supplementation and intramuscular injections of vitamin B12 will increase serum B12 levels. While these are possible treatments for elevating B12, studies also suggest that patient adherence to oral medications is not adequate (50%) and studies regarding intramuscular injection list the pain involved with injection, and cost of the injections as deterring elements for this type of treatment. This particular route of administration (nebulization) has not been confirmed as a viable option for elevating serum vitamin B12 levels.

CHAPTER TWO

REVIEW OF LITERATURE

INTRODUCTION

There are many studies regarding vitamin B12 and the importance of maintaining adequate levels of vitamin B12 to prevent conditions such as pernicious (megaloblastic) anemia, neurologic deficits, and psychoses as well as ensure proper DNA synthesis, red blood cell production, fatty acid synthesis and conversion of homocysteine into methionine. There are two common ways to increase vitamin B12 levels: daily oral supplementation and weekly/monthly intramuscular injections. The focus of this study is to determine if a different route of administration of vitamin B12 will increase serum vitamin B12 levels.

This review of literature included database (PubMed, EBSCO, Science Direct) searches using the following key words singularly or in combination: vitamin B12, cobalamin, cyanocobalamin, metformin, elderly, psychosis, DNA, anemia, cognitive, atrophic gastritis, adherence, intramuscular injection, oral, supplementation, development, gastric bypass surgery, proton pump inhibitors, H2 antagonists, homocysteine, dementia, and/or vegetarian. The review of literature has been subdivided into the following sections: 1) normal utilization of vitamin B12 2) vitamin B12 deficiencies 3) vitamin B12 intramuscular injection and oral supplementation and 4) medication adherence.

NORMAL UTILIZATION OF VITAMIN B12

Normal absorption of vitamin B12 in humans is typically dependent upon the ileum absorbing intrinsic factor-bound vitamin B12. There is evidence suggesting that some vitamin B12 may be absorbed independent of the normal intrinsic factor bound vitamin B12 and that absorption may happen by passive diffusion when taking large oral doses of vitamin B12. It has

been found that approximately 1% of orally administered doses of vitamin B12 are absorbed by passively diffusing into the blood stream (Berlin, Berlin, & Brante, 1968). Once vitamin B12 has been absorbed, it is used in two enzymatic reactions. First, vitamin B12 is utilized in the conversion of methylmalonic acid into succinyl-CoA and second, in the conversion of homocysteine into methionine.

VITAMIN B12 DEFICIENCIES

According to Herbert (1994) there are 4 stages of B12 insufficiency: serum depletion, cell depletion, biochemical deficiency defined as elevated levels of homocysteine and MMA, and finally classic signs and symptoms of clinical deficiency (Herbert, 1994). According to the website for the Centers of Disease Control (CDC), the most frequently reported threshold value for low vitamin B12 is 200 pg/mL (148 pmol/L) (Centers of Disease Control and Prevention, 2009). Although vitamin deficiencies are relatively uncommon in the Western world, it is estimated that 10% to 15% of individuals over the age of 60 years may suffer from B12 deficiency (Hanna, Lachover, & Rajarethinam, 2009). A study performed at the Jordan University hospital revealed 78.8% of the 838 persons studied were either hypovitaminosis (34.2%, 180-300 pg/mL) or vitamin b12 deficient (44.6% <180 pg/mL) (Barghouti, Younes, Halaseh, Said, & Ghraiz, 2009). Another study performed on a Brazilian population found that 7.2 % of the participants aged 60 years or more and 6.4 % of the participants aged 30-59 years had vitamin B12 levels <200 pmol/L or <271 pg/mL (Xavier, Costa, Annichino-Bizzacchi, & Saad, 2010). The CDC also reports that unpublished data from the National Health and Nutrition Examination Survey (NHANES) 2001-2004 stratified by age have estimated that 1 (3.2%) of every 31 adults 51 years of age or older in the United States will have a low vitamin B12 serum level (≤ 200 pg/mL). Most of these people are ambulatory and do not have overt symptoms of

vitamin B12 deficiency (Centers for Disease Control and Prevention, 2009). Oh and Brown (2003) listed the following clinical manifestations of vitamin B12 deficiency: megaloblastic anemia, pancytopenia (leukopenia, thrombocytopenia) paresthesias, peripheral neuropathy, combined systems disease (demyelination of dorsal columns and corticospinal tract), irritability and personality change, mild memory impairment and dementia, depression, psychosis, and possible increased risk of myocardial infarction and stroke and stroke (Oh & Brown, 2003).

Vitamin B12 and the elderly

The United States population has grown 9.7% since 2000 and the population of people age 65+ years has grown 15.1% (United States Department of Commerce, 2011). Vitamin B12 deficiency is a common in elderly people and age related changes can affect vitamin B12 status. Epidemiological studies show a prevalence of cobalamin deficiency of around 20% (between 5% and 60%, depending on the definition of cobalamin deficiency used in the study) in the general population of industrialized countries (Andres et al., 2004). In a study of the Framingham elderly, the prevalence of cobalamin deficiency was > or = 12% in a large sample (*n*=548) of free-living elderly Americans (Lindenbaum, Rosenberg, Wilson, Stabler, & Allen, 1994). A study of older hospital patients considered them to be cobalamin deficient when one of three criteria was fulfilled: 1) hematological or metabolic abnormalities compatible with cobalamin deficiency; 2) cobalamin malabsorption or atrophic gastritis; 3) a response to cobalamin supplementation. The study of 28 patients found that all patients were considered cobalamin deficient based on the previously discussed criteria (van Asselt et al., 2000). In a cross sectional study conducted from 2008 to 2010 in a population over 65 years of age, vitamin B12 deficiency was found in 16.5% of the sample (vasquez-Pedrazuela et al., 2012). Johnson et al. (2010) performed a study on African-American and white octogenarians and centenarians in Georgia.

The study consisted of 80 men and women octogenarians and 231 centenarians. After they excluded those already receiving vitamin B12 injections (n = 17) they found vitamin B12 deficiency in 35.3% of the centenarians and 22.8% of the octogenarians. Their conclusions were that both centenarians and octogenarians are at a high risk for vitamin B12 deficiency and given the numerous potential adverse consequences of poor vitamin B12 status, efforts are needed to ensure vitamin B12 adequacy in older adults (Johnson et al., 2010). Due to the prevalence of vitamin B12 deficiency in older adults and the neuropsychiatric, neurologic and hematologic problems that occur due to B12 deficiency it is important to monitor these levels in elderly individuals. Allen and Casterline (1994) suggest that current serum cut offs for diagnosing vitamin B12 deficiency are too low and should be raised to 190 pg/mL (L. Allen & Casterline, 1994).

Vitamin B12 and Dementia

According to the World Health Organization dementia is a syndrome in which there is deterioration in memory, thinking, behavior and the ability to perform everyday activities that effects 35.6 million people world-wide with 7.7 million new cases every year. Dementia mainly affects older people and it is not a normal part of ageing. Dementia affects each person differently, depending upon the impact of the disease and the person's personality before becoming ill (World Health Organization, 2012).

Vitamin B12 deficiency chiefly occurs in the elderly, predominantly the result of malabsorption, gastric atrophy, or ileal disease (Clark, 2008). Depression, dementia, and mental impairment are often associated with vitamin B12 and folate deficiency, especially in the elderly (Hanna et al., 2009). Older adults presenting with low vitamin B12 rarely have classical features of macrocytic anemia and neuropathy, rather more commonly present with non-specific

symptoms of fatigue and cognitive impairment that can be attributed to 'old age' (Clark, 2008). It is less well known that psychiatric symptoms may be the first symptoms of B12 deficiency and may happen before hematological and neurological symptoms by a long period (van Tiggelen, Peperkamp, & Tertoolen, 1983). Vitamin B12 deficiency is a slowly progressive process where most cases are now detected at an earlier stage when clinical manifestations are often subtle and highly variable and neuropsychiatric symptoms may occur in the absence of hematological signs (Clark, 2008). Vitamin B12 and folate are connected with synthesis of monoamines such as dopamine and serotonin and are involved in single carbon transfer methylation reactions connected with the production of these neurotransmitters that are implicated in the pathophysiology of neuropsychiatric disorders such as depression and psychosis (Hanna et al., 2009).

A randomized controlled trial of 409 participants with mild to moderate Alzheimer's disease was performed to investigate high dose B vitamin (folic acid, B6, and B12) supplementation and cognitive decline in Alzheimer's disease. The authors found that daily high dose supplements (folate 5mg, vitamin B6 25 mg, and vitamin B12 1 mg) does not slow cognitive decline in individuals with mild to moderate Alzheimer's disease (Aisen et al., 2008).

A double-blind randomized placebo controlled trial of 266 subjects receiving a daily dose of 0.8 mg folic acid, 0.5 mg vitamin B12 and 20 mg vitamin B6 was performed. It was found that the B vitamin supplements appeared to slow cognitive and clinical decline in people with mild cognitive impairment, in particular in those with elevated homocysteine (de Jager, Oulhaj, Jacoby, Refsum, & Smith, 2012).

According to Geldmacher (2004), the American Academy of Neurology practice guidelines recommends vitamin B12 level determination as part of the routine workup for

dementia because although reversible causes of dementia are infrequent and treatment is unlikely to fully reverse the dementia, appropriate treatment may reduce excess disability (Geldmacher, 2004).

Vitamin B12 and Atrophic Gastritis

The most common diseases of the stomach in elderly individuals are atrophic gastritis and peptic ulcer disease (PUD). Atrophic gastritis (AG) is a histopathological entity that is characterized by chronic inflammation of the gastric mucosa with loss of gastric glandular cells and replacement by intestinal-type epithelium, pyloric-type glands, and fibrous tissue (Dai, Tang, & Zhang, 2011). Atrophic gastritis is significantly associated with helicobacter pylori infection and reduced acid secretion. Helicobacter pylori is the main cause of chronic gastritis (Goodwin, Mendall, & Northfield, 1997). Hyposecretion of gastric acid reduces the absorption of vitamin B12, iron, and calcium, and these deficits can lead to megaloblastic or iron-deficiency anemia and a higher frequency of osteoporosis (Grassi et al., 2011). Atrophic gastritis, either related or unrelated to helicobacter pylori infections, can cause a food-cobalamin malabsorption syndrome because of the inability to release the vitamin B12 from food or its binding proteins (Carmel, 2000; Dali-Youcef & Andres, 2009).

A study performed in Finland (N = 4,256, average age 56 years, range 18-92 years) found that 3.5% (150 individuals) of the sample had atrophic corpus gastritis. In the age group of 70 or over, the prevalence of atrophic corpus gastritis increased to 8% (62 individuals). The author's conclusion stated that advanced atrophic corpus gastritis is a common disease among Finnish adults and remains to be undiagnosed in most under the current healthcare practice (Telaranta-Keerie, Kara, Paloheimo, Harkonen, & Sipponen, 2010). A Japanese study of 2,633 subjects at a "human dry dock" (a medical health checkup that promotes health by early detection of chronic

diseases and their risk factors) found that 531 (20.2%) were diagnosed with atrophic gastritis. After adjusting for age, sex, obesity, hypertension, diabetes mellitus, dyslipidemia, hyperuricemia, and habits of smoking and drinking the authors found that atrophic gastritis is an independent risk factor for coronary artery disease (Senmaru et al., 2012). A study performed on 31 patients with atrophic gastritis were compared with 28 patients with non-atrophic gastritis found that atrophic gastritis, rather than H. pylori infection per se, may be a contributing factor to hyperhomocysteinemia, possibly via vitamin B12 malabsorption (Santarelli et al., 2004). A study performed on nineteen volunteers, either healthy or with low cobalamin levels, were prospectively studied without prior knowledge of their absorption or gastric status. All of the subjects underwent prospective assessment of food cobalamin absorption by the egg yolk cobalamin absorption test, endoscopy, histological grading of biopsies from six gastric sites, measurement of gastric secretory function, assay for serum gastrin and antiparietal cell antibodies, and direct tests for Helicobacter pylori infection. The authors' conclusion of the study were that severe food cobalamin malabsorption can occur in patients who do not have atrophic gastritis of the oxyntic mucosa or achlorhydria, and that food cobalamin malabsorption should never be automatically equated with atrophic gastritis (Cohen, Weinstein, & Carmel, 2000).

Vitamin B12 and Gastric Bypass Surgery

Gastric bypass surgery is a procedure to make the stomach smaller and cause food to bypass part of the small intestine (Weight Loss Surgery Center, 2011). It is estimated that approximately 113,000 to 120,000 gastric bypass surgeries take place each year (Diseases, 2010; Livingston, 2010). Donadelli et al. (2011) indicate that this procedure promotes early satiety and decreases food intake but warn that it also may cause altered nutrient levels with the most

frequent deficiencies being iron, vitamin B1, vitamin B12, folic acid, vitamin D and calcium (Donadell et al., 2011). After gastric bypass surgery, vitamin B12 deficiency is due to a failure of the separation of vitamin B12 from haptocorrin and subsequent failure of absorption of B12 in the presence of intrinsic factor (Rhode et al., 1995). Although oral supplementation containing the RDA for micronutrients can prevent abnormal blood indicators of most vitamins and mineral, it is insufficient to maintain normal plasma B12 levels in about 30% of gastric bypass patients (Provenzale et al., 1992). Up to 37% of patients who are prescribed a multivitamin after surgery still develop vitamin B12 deficiency (Brethauer, Chand, & Schauer, 2006). Williams, Cooper, Richmond and Schauer (2008) indicated that vitamin B12 supplementation is recommended for all patients after bariatric surgery because deficiency is common and that patients with relatively mild malabsorption can maintain B12 levels with oral supplementation but many patients require lifelong subcutaneous injections (Williams, Cooper, Richmond, & Schauer, 2008). Rhode et. al (1995) found that at least 350 micrograms of oral dose crystalline vitamin B12 after gastric bypass surgery corrected low serum vitamin B12 levels in 95% of patients (N=102).

In a study of 348 patients who received Roux-en-Y gastric bypass surgery it was found that preoperative levels of vitamin B12 were higher that postoperative levels. It was also found that taking multivitamin supplements resulted in lower incidence of folate deficiency but did not prevent vitamin B12 deficiency and oral supplementation of vitamin B12 corrected the vitamin B12 deficiencies in 81% of the cases. The researchers' conclusions were that a lack of symptoms of vitamin B12 deficiency suggested that the deficiencies are not clinically important after Roux-en-Y surgery (Brolin et al., 1998). In a prospective study following 150 patients receiving gastric exclusion surgery for morbid obesity, anemia developed in 36.8% of the patients in an average of 20 months after surgery. The authors also noted that a low serum

vitamin B12 concentration (150 – 230 pg/mL) was found in 70.1% of the total population and vitamin B12 deficiency (< 150 pg/mL) was found in 39.6% of the total population (Amaral, Thompson, Caldwell, Martin, & Randall, 1985).

Vitamin B12 and Pernicious Anemia

Pernicious anemia is a macrocytic anemia due to vitamin B12 deficiency and is most frequent among elderly patients (Fernandez-Banares, Monzon, & Forne, 2009). This deficiency is the result of a lack of intrinsic factor, a protein that binds dietary vitamin B12 and promotes its transport to the terminal ileum for absorption. The deficiency of intrinsic factor is a consequence of an autoimmune atrophic gastritis (Andres & Serraj, 2012), which results in the destruction of the oxyntic mucosa, and thus, the loss of parietal cells, which normally produce chlorhydric acid as well as intrinsic factor (Lahner & Annibale, 2009). The clinical presentation of pernicious anemia is insidious for various reasons, including the storage capacity of vitamin B12 in the liver. Patients are often unaware of their anemia related symptoms because they have become used to them. Patients with pernicious anemia may seek medical advice for a symptom such as weakness or decreased mental concentration rather than pernicious anemia itself, in which case a complete blood count may reveal the underlying disease (Lahner & Annibale, 2009).

Vitamin B12 and Homocysteine

Homocysteine is a sulfur amino acid that is at the crossroads of two pathways: remethylation to methionine which requires folate(vitamin B9) and cobalamin(vitamin B12) and transsulfuration to cystathionine, which requires pyridoxal-5`-phospate(vitamin B6) (Selhub, 1999). In the remethylation of homocysteine to methionine, homocysteine acquires a methyl group from N-5-methyltetrahydrofolate or from betaine. The reaction with N-5-methyltetrahydrofolate occurs in all tissues and is dependent upon the presence of vitamin B12,

whereas the reaction with betaine is confined to the liver and is independent of vitamin B12 (Selhub, 1999).

Methionine synthase is one of two known vitamin B12 dependent enzymes (the other is L-methylmalonyl-CoA mutase). Methionine synthase catalyzes the conversion of 5-CH3-tetrahydrofolate and homocysteine to tetrahydrofolate and methionine (R. Allen, Stabler, Savage, & Lindenbaum, 1993). Allen et al. (1993) go on to explain that the conversion of homocysteine into methionine is not vitamin B12 dependent. The enzyme betaine-homocysteine methyl-transferase catalyzes a reaction in which a methyl group from betaine is transferred to homocysteine to form N,N-dimethylglycine and methionine. The authors continue to explain that serum betaine levels are maintained in the majority of patients with either vitamin B12 or vitamin B9 deficiency. The authors state: "This observation provides additional support for the concept that an inability to provide adequate amounts of methionine occurs rarely in either cobalamin or folate deficiency (R. Allen et al., 1993).

Methylcobalamin is one co-factor in the conversion of homocysteine into methionine. Methyltransferase is an enzyme that removes a methyl group from a donor and transfers it to an acceptor. In the case of vitamin B12, methylcobalamin donates its methyl group along with 5-methyltetrahydrofolate to homocysteine, converting homocysteine into methionine. This reaction occurs in the cytoplasm and is catalyzed by methionine synthase (Kapadia, 1995). Homocysteine is a risk factor for cardiovascular disease (Bonaa et al., 2006). Lower homocysteine levels are associated with lower rates of coronary heart disease and stroke (Lonn et al., 2006). Observational data suggest that even mild-to-moderate elevations in homocysteine levels increase cardiovascular risk and such increases are common and can be easily corrected with safe and inexpensive therapy (Lonn et al., 2006).

Daily supplementation of 0.5 to 5.0 mg of folic acid typically lowers plasma homocysteine levels approximately 25% and vitamin B12 supplementation of at least 0.4 mg daily further lowers homocysteine by about 7% (Lonn et al., 2006). A meta-analysis investigating dose-dependent effects of folic acid on blood concentrations of homocysteine included the effects seen by adding vitamin B12 to folic acid supplementation. The results of the meta-analysis were that the addition of vitamin B12 (median dose of .4 mg) reduced homocysteine levels by approximately 7%. After standardization to pretreatment concentrations of homocysteine and folate to equal proportions of men and women, the addition of vitamin B12 to a folic acid dose of 5 mg changed the reduction in homocysteine from 25% to 30%. They also found that the addition of vitamin B12 to median folic acid doses of 0.2, 0.4, 0.8, and 2.0 mg/d resulted in homocysteine reductions of 19%, 25%, 29% and 28% respectively while data for examination of differences in the effect of different doses of vitamin B12 were insufficient. The conclusion of the article stated that little further homocysteine reduction occurs by increasing the dose of folic acid above 0.8 mg/d but the combined effect of adding vitamin B12 with folic acid will achieve a greater reduction in plasma homocysteine concentration that folic acid alone (Homocysteine Lowering Trialists' Collaboration, 2005).

Vitamin B12 and Exercise

Lukaski (2004) reports that interest in vitamin B12 among physically active individuals stems from its vital role in erythropoiesis (red blood cell production). He goes on to indicate that athletes on energy restrictive diets and strict vegetarians are at risk for vitamin B12 depletion and that supplemental B12 does not benefit performance unless a nutritional deficit is present (Lukaski, 2004). Beals and Manore (1998) found that female athletes with subclinical eating disorders who had intakes of energy, protein, carbohydrate and certain micronutrients below

recommended levels did not negatively affect the athletes micronutrient status, which may be explained, in part, by their used of vitamin/mineral supplements (Beals & Manore, 1988). Most research studies regarding vitamin B12 do not look solely at vitamin B12 alone, but involve other substances as well, some of which include vitamins B6, B9, B1 and B2 as well as homocysteine and methionine. It is generally accepted that vitamin B12 aids in the formation of red blood cells, DNA synthesis and the maintenance of the myelin sheath surrounding some nerve fibers (Woolf & Manore, 2006). A deficiency of vitamin B12 can lead to a condition known as pernicious anemia. Pernicious anemia is a megaloblastic form of anemia that is characterized by a larger than normal size of the red blood cell (Woolf & Manore, 2006). As stated previously in chapter 1, symptoms of vitamin B12 deficiency include: megaloblastic anemia, fatigue, weakness, constipation, loss of appetite, weight loss, numbness and tingling of hands and feet, difficulty maintaining balance, depression, confusion, dementia, poor memory and soreness of the mouth or tongue (National Institutes of Health, 2012).

Some studies have investigated the importance of vitamin B12 as it relates to homocysteine levels in individuals who exercise. The importance of both vitamins B9 and B12 are in the reduction of homocysteine levels is generally accepted, however the opinions regarding the importance of vitamin B12 alone in the reduction of homocysteine levels needs further study. A study performed by Herrman et al. (2003) investigated the influence of volume-oriented training and high intensity interval training on serum homocysteine and its co-factors in young healthy swimmers. Their study compared homocysteine, vitamin B12, vitamin B6, vitamin B9, and methylmalonic acid (MMA) before and after 3 weeks of volume oriented training (30 km per week) and high intensity interval training (20 km per week) in 20 young (age 16 +/- 2 years) female swimmers. They found that training increased homocysteine levels in

both the high intensity interval and the volume oriented training groups, with the elevation remaining during the recovery period (1 week). They found that vitamin B12 was unchanged after training in both training groups but decreased during the recovery period. During their discussion they indicated that: "there is no proven explanation for an exercise induced increase in serum homocysteine. The negative correlation between the change in homocysteine and the vitamin B12 baseline levels suggests that the vitamin B12 dependent remethylation of homocysteine might be an important regulating mechanism" (Herrmann, Wilkinson, et al., 2003).

Konig et al. (2003) investigated the influence of extensive endurance training and acute intense exercise on plasma concentrations of total homocysteine, vitamin B12 and vitamin B9. Their study included 42 well-trained male triathletes. They took blood samples before and after a 30-day endurance-training period. They also took blood samples at 1 hour and 24 hours after a competitive exercise (sprint triathlon). Their analysis showed that exercise induced increase in the homocysteine levels was dependent on baseline levels of folate and their training volume but not on vitamin B12. They concluded that although the role of plasma B9 and B12 levels in reducing homocysteine levels is widely accepted, vitamin B12 was not associated with homocysteine at any point during their study (Konig et al., 2003).

Herrmann, Schorr, et al., (2003) performed a study that investigated 100 recreational athletes who participated in a marathon race (N =46), a 100 km run (N=12), or a 120 km mountain bike race (N=42). They took blood samples before, 15 minutes after, and 3 hours after the races. They found that marathon running induced a homocysteine increase of 64%, while mountain biking and 100 km running had no significant effect on homocysteine levels. They stated that the data demonstrate that acute endurance exercise may induce a relevant

homocysteine increase that lasts up to 24 hours after the end of exercise. This increase depends strongly upon the type and duration of exercise and possibly also from the years of regular training. Another finding of the study was the high frequency of elevated pre-race homocysteine levels in recreational athletes (23 of 100). The researchers indicated that since none of the athletes performed strenuous training during the 48 hours before the race, previous exercise as a possible reason for elevated homocysteine levels at baseline did not seem probable. Instead, they indicated that relatively low folate and vitamin B12 levels were much more likely the cause. They found that in the 23 athletes with elevated homocysteine levels, their median folate and vitamin B12 levels were distinctly lower than comparable controls (Herrmann, Schorr, et al., 2003).

A study performed by van Schoor et al., (2012) examined a cross-sectional and longitudinal association between homocysteine, vitamin B12 and physical performance in older persons. This study investigated persons > 65 years of age. They collected blood in 1995/1996 (n= 1352) and tested physical performance in 1995/1996 and 1998/1999 using: the walking test, the chair stands test and the tandem test (N = 901 in 1995/1996) (N = 1155 in 1998/1999). Their results indicated that after adjusting for confounding that women in the highest quartile of homocysteine had a significantly lower physical performance that did those in the lowest quartile in the cross-sectional analyses. This association was borderline statistically significant in the longitudinal analysis. After adjusting for serum vitamin B12 levels, both associations were statistically significant. For vitamin B12 in women, and for homocysteine and vitamin B12 in men, the observed associations were less consistent. The authors concluded that high plasma homocysteine is an independent risk factor for lower physical performance in older women and

that the association between vitamin B12 and physical performance is less clear (van Schoor et al., 2012).

Joubert and Manore (2008) investigated the relationship between exercise and homocysteine to determine whether plasma homocysteine values, independent of plasma B-vitamin concentrations were higher in active that less active subjects. They utilized 38 males and 38 females who reported being physically active for the last 5 years. They determined dietary intakes of B-vitamins based on using 7-day weighted food records. Their study found that homocysteine was not different between physical activity levels in non-supplementing adults unless the physical activity was high (90% VO2 max) (Joubert & Manore, 2008).

A placebo-controlled study from 1955 investigated the effects of vitamin B12 supplementation on physical fitness and growth of young boys (aged 12-17). The subjects were residents of a state correctional institution. The activities each participated in were rigidly regimented, each receiving an hour of physical education daily. The authors concluded that the vitamin B12 supplements had no effect on physical fitness and growth. They found no differences on the Harvard Step Test score, half-mile run time, height, weight or combination of age, height, and weight (Montoye, Spata, Pinckney, & Barron, 1955).

Vitamin B12 and Vegetarians

According to the Vegetarian Resource Group, approximately 3% or 6-8 million people in the United States are classified as vegetarian (never eat meat) (Group, 2009). The most common explanations for poor vitamin B12 status are low dietary intake and malabsorption (L. Allen, 2008). A dietary deficiency of vitamin B12 can result from a strict vegan diet since there is not vitamin B12 synthesized by any plant, nor does the plant need, use, or store vitamin B12 (Herbert, 1994). Vegans, and to a lesser extent vegetarians, have low average circulating

concentrations of vitamin B12 (Gilsing et al., 2010). Gilsing et al. (2010) reported that their cross-sectional analysis revealed that mean serum B12 was highest among omnivores (380 pg/mL), intermediate among vegetarians (246 pg/mL), and lowest among vegans (165 pg/mL), with vitamin B12 deficiency defined as <159 pg/mL. Dong and Scott (1982) reported that 92% of vegans, 64% of lactovegetarians, 47% of lacto-ovovegetarians, and 20% of semi-vegetarians have serum vitamin B12 levels less than 200 pg/mL (Dong & Scott, 1982). Allen (2008) indicated that it has long been known that strict vegans are at risk for vitamin B12 deficiency, and that evidence shows that low intakes of animal-source foods, such as some lacto-ovo vegetarians and many less industrialized countries cause vitamin B12 depletion. Pongstaporn and Bunyaratavej (1999) reported that hematological parameters of hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cells, neutrophils, serum ferritin and serum vitamin B12 in vegetarians were significantly lower than the controls (Pongstaporn & Bunyaratavej, 1999). Woo et al (1998) reported that serum B12 levels were below the reference range (< 203 pg/mL) in 54% of vegetarians (Woo, Kwok, Ho, Sham, & Lau, 1998).

Vitamin B12 and Metformin

Metformin, a biguanide, was introduced in the United Kingdom in 1958, in Canada in 1972, and in the United States in 1995 (Mahajan & Gupta, 2010). Metformin is one of two oral medications listed by the World Health Organization for the treatment of diabetes (World Health Organization, 2011) and because of this, metformin has become the most widely used anti-diabetic drug (Mahajan & Gupta, 2010). Mahajan and Gupta (2010) also report that within the first 10–12 years after it came into use, it became evident that long-term metformin therapy causes vitamin B12 malabsorption.

Patients receiving metformin have diminished B12 absorption and low serum vitamin B12 levels. According to Bauman et al (2000) approximately 10% but up to 30% of patients taking metformin on a continuous basis have evidence of reduced B12 absorption. Metformin is known to have an effect on calcium dependent membrane action. The B12-intrinsic factor complex uptake by ileal cells is known to be calcium dependent (Bauman, Shaw, Jayatilleke, Spungen, & Herbert, 2000). The study performed by Bauman et al (2000) investigated 21 people with type 2-diabetes. The results of their study showed that calcium supplementation reversed the metformin-induced serum and holo-transcobalamin II vitamin B12 levels. A study in Brazil was performed to evaluate the presence of vitamin B12 deficiency and factors associated with serum vitamin B12 levels in a sample (n = 144) of metformin-treated diabetic patients. The results showed that 6.9% of the sample had low serum B12 (< 169 pg/mL) and that 36.8% of the sample was possibly low (169 pg/mL – 338 pg/mL) (Nervo et al., 2011). The conclusion of their study was that present findings suggest a high prevalence of vitamin B12 deficiency in metformin-treated diabetic patients and that older patients; patients in long term treatment with metformin and low vitamin B12 intake are probably more prone to this deficiency (Nervo et al., 2011). In 1971 a study was done at Royal Victoria Hospital on all diabetic patients who had been taking metformin for more than two years. Of the 71 patients investigated 21 were shown to have vitamin B12 malabsorption and four of these 21 were found within six years of beginning metformin therapy (Tomkin, Hadden, Weaver, & Montgomery, 1971).

Vitamin B12 and Proton Pump Inhibitors / H2 Receptor Antagonists

H2 receptor antagonists (H2RAs) are a classification of drugs that block histamine receptors of the parietal cells blocking the action of histamine, thereby decreasing the acid production of the parietal cells. Proton pump inhibitors (PPIs) are a class of medication that

work by blocking the hydrogen/potassium adenosine triphosphate enzyme (H+/K+ ATPase) of the parietal cells of the stomach.

Studies have reported inconclusive evidence that taking H2RAs or PPIs will cause a vitamin B12 deficiency. Force and Nahata (1992) reported that H2RAs have not been shown to decrease intrinsic factor secretion but that studies have demonstrated a reduction in food-bound vitamin B12 absorption secondary to decreased acid secretion in patients taking the H2RA medication (Force & Nahata, 1992). They also note that H2RAs have the potential to cause vitamin B12 deficiency and this may be important in persons who have inadequate stores of vitamin B12 (Force & Nahata, 1992). Valuck and Ruscin (2004) showed that controlling for age, gender, multivitamin use, H. pylori infection, chronic (12 months) current use of H2RA or proton pump inhibitors (PPIs) was associated with a significantly increased risk of vitamin B12 deficiency, also noting that no association between short term current use of H2RA/PPIs and vitamin B12 deficiency (Valuck & Ruscin, 2004).

The use of H2RAs and/or PPIs may impair absorption of protein-bound dietary vitamin B12 and could contribute to the development of vitamin B12 deficiency with prolonged use (Ruscin, Page, & Valuck, 2002). In a review of effects of long term acid suppression, Laine et. al. (2000) reported concern that antisecretory therapy may result in vitamin B12 malabsorption (Laine, Aiinen, McClain, Solcia, & Walsh, 2000). Marcuard, Albernaz, and Khazanie (1994) found that omeprazole therapy acutely decreased cyanocobalamin absorption in a dose dependent manner (Marcuard, Albernaz, & Khazanie, 1994). Absorption of vitamin B12 was decreased from 3.2% to .9% in participants taking 20mg of omeprazole daily and absorption was decreased from 3.4% to 0.4% in participants taking 40 mg of omeprazole daily (Marcuard et al., 1994). A study evaluating absorption of protein-bound and unbound cyanocobalamin before and

during treatment with omeprazole found that the absorption of protein-bound, but not unbound, cyanocobalamin is decreased with omeprazole but no change in serum cobalamin levels were observed in patients with omeprazole treatment for gastroesophageal reflux disease (GERD) for up to seven years (Schenk, Festen, Kuipers, Klinkenberg-Knol, & Meuwissen, 1996).

An investigation on the effects of hypochlorhydria and acidic drink ingestion on protein bound vitamin B12 in the elderly revealed that omeprazole causes protein-bound vitamin B12 malabsorption and the ingestion of an acidic drink improves protein-bound vitamin B12 absorption (Saltzman et al., 1994). Overall, proton pump inhibitors are a safe class of medications to use long term in persons in whom there is a clear need for the maintenance of extensive acid inhibition (Thompson, Suave, Kassam, & Kamitakahara, 2010).

VITAMIN B12 INTRAMUSCULAR INJECTION AND ORAL SUPPLEMENTATION

There are two common routes of administration to elevate serum vitamin B12 levels in people; intramuscular injection and daily oral supplementation. One study compared oral supplementation (2 mg) versus intramuscular injection (1 mg). The intramuscular injection group received 9 injections over the course of 60 days and the oral supplementation group took 2 mg of cyanocobalamin daily for 120 days. The pretreatment values of serum cobalamin for the two groups (oral and injection) were 93 pg/mL and 95 pg/mL respectively. After their treatments the mean serum cobalamin levels were 1005 pg/mL (oral) and 325 pg/mL (Kuzminski, Del Giacco, Allen, Stabler, & Lindenbaum, 1998). In a study on the effect of small doses (10 microgram and 50 microgram) of oral B12 supplementation it was found that the 50 microgram group was had a significantly greater increase in serum vitamin B12 levels compared to the control group but the 10 microgram group was not significantly different than the controls (Seal, Metz, Flicker, & Melney, 2002). In a study of patient perspectives regarding vitamin B12

supplementation of oral therapy versus injection therapy 73% of the participants were willing to try oral B12 as their route of administration for vitamin B12 supplementation. Of those 73% who were willing to switch to oral B12, 71% wished to permanently switch from injection B12 to oral B12. The major factors associated with permanent switching included believing that the frequent visits to their health care provider as well as travel/parking costs were disadvantages of injection B12. Another factor associated with switching was potential complications associated with injections (Kwong, Carr, Dhalla, Tom-Kun, & Upshur, 2005). One last factor regarding vitamin B12 supplementation is cost. Lane and Rojas-Fernandez (2002) reported on wholesale costs for vitamin B12 supplementation. They reported that average wholesale price for one hundred 1- milligram oral tablets is approximately $4.00 and average wholesale price of 1 milligram injection of vitamin B12 is approximately $0.11 however the physician visit for an injection will cost between $20 and $30 and home-nursing service will cost between $60 and $100 per injection (Lane & Rojas-Fernandez, 2002).

NEBULIZED GLUTATHIONE AND MAGNESIUM

Other items that have found use in nebulized form include glutathione and magnesium. Glutathione (GSH) is a non-essential antioxidant compound comprised of cysteine, glycine, and glutamic acid. GSH is the major antioxidant made within the human body. Its role in the human body is to donate a hydrogen ion to neutralize free radicals and oxidative compounds. Nebulized glutathione has been shown to be an effective treatment for a variety of pulmonary diseases and respiratory-related conditions with very serious and difficult-to-treat diseases (e.g., cystic fibrosis, idiopathic pulmonary fibrosis) yielded benefits from this novel treatment (Prousky, 2008).

Nebulized and/or intravenous magnesium is currently being utilized in some persons with chronic obstructive pulmonary disease and asthma. Studies are inconclusive with regards to this form of treatment. Scarfone et al., 2000 investigated magnesium use in the emergency department treatment of children with asthma. Their study was a randomized, double-blind, placebo controlled trial of 54 children aged 1 to 18 years who presented to the emergency department with a moderate to severe exacerbation of asthma. The patients were given a nebulized treatment of albuterol (0.15 mg /kg) and methylprednisolone (1 mg/kg). Then the patients were randomly assigned to receive either 75 mg/kg of magnesium sulfate (maximum of 2.5 g) or placebo. The outcome of the study was degree of improvement as assessed by Pulmonary Index scores over 120 minutes. Secondary outcomes included hospitalization rates and time required to meet discharge criteria. Their results indicated a mean change in pulmonary index score from baseline to 120 minutes was 2.83 for the magnesium group compared to 2.66 for the placebo group. Eleven of the 24 magnesium-treated group (46%) were hospitalized compared to 16 of the 30 placebo group (53%). There was no statistical difference between groups with respect to the time required to meet discharge criteria. The authors conclusion was that the routine administration of high dose magnesium to moderate to severely ill children with asthma, as an adjunct to initial treatment with albuterol and corticosteroids was not efficacious (Scarfone et al., 2000).

Nouira et al., (2012) performed a randomized trial investigating magnesium sulfate versus ipratropium bromide in chronic obstructive pulmonary disease exacerbation. The study consisted of two groups: a nebulized ipratropium bromide group (n = 62) and a combined nebulized and intravenous bolus of magnesium sulfate group (n = 62). The drugs were administered at 30 minute intervals for two hours. The primary outcomes investigated were

hospital admission, endotracheal intubation and hospital death rates. The secondary outcomes investigated were improvement in peak expiratory flow, dyspnea score, and arterial blood gas changes within 3 hours. The study showed no significant difference in primary outcomes between each group. Patients who were give ipratropium bromide had an average of 32 L greater improvement in peak expiratory flow rate when compared with magnesium sulfate at 180 minutes. There was a significant reduction in partial pressure of carbon dioxide (PaCO2) compared with baseline values in the ipratropium bromide group but not in the magnesium sulfate group. There was a trend toward a decrease in dyspnea score in both groups although it was statistically nonsignificant. Also, the improvement in peak expiratory flow rate and arterial blood gas favored the nebulized ipratropium bromide group and there was a nonsignificant difference between both drugs with regard to hospital admission, intubation, and hospital death rates in patients with chronic obstructive pulmonary disease treated in the emergency department for acute exacerbation (Nouira et al., 2012).

Chande and Skoner (1992) performed a randomized, double-blind, crossover clinical trial of nebulized magnesium sulfate to reverse bronchospasm in asthmatic patients testing each subjects forced expiratory volume in one second (FEV1). They had 10 patients (21-37 years) who withheld their asthma medications for 24 hours. The subjects then underwent bronchial methacholine challenge to produce bronchospasm. They then received one of three different nebulized treatments: 2.5 mg albuterol in 3 mL saline, 3 mL magnesium sulfate or 3 mL normal saline. Their average results were that nebulized albuterol increased FEV1 56%, normal saline increased FEV1 29% and magnesium sulfate increased FEV1 12%. Their conclusion was that nebulized magnesium sulfate has a minimal bronchodilatory effect in asthmatic patients with methacholine-induced bronchoconstriction (Chande & Skoner, 1992).

In a systematic review of literature, Blitz et al. (2005) concluded that nebulized magnesium sulfate appears to be a safe and effective treatment to administer to patients experiencing asthma exacerbations. He indicates that it has been demonstrated that therapy with magnesium sulfate and beta-2 agonists improved lung function when compared to therapy utilizing beta-2 agonists alone and that treatment with nebulized magnesium sulfate should be considered as an addition to inhaled beta-2 agonist therapy in patients experiencing asthma exacerbations (Blitz et al., 2005).

MEDICATION ADHERENCE

According to the World Health Organization the definition of adherence is: the extent to which a person's behavior – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider (World Health Organization, 2003). Recent studies on patient compliance with lifestyle changes and on adherence to prescribed medication have produced alarming findings. Over 50% of patients, on average, choose to abandon the treatment they have been prescribed (Fuster, 2012; Laba, Brien, & Jan, 2012). Rates of non-adherence are estimated to be greater still within marginalized groups and in developing countries. Interventions to promote adherence have been shown, at best, to be of moderate effectiveness and cost effectiveness (Laba et al., 2012). Meddings et al. (2012) found that with regards to physician assessment of patients with high blood pressure, providers overestimated adherence with antihypertensive medications, and were still less accurate in identifying cases of significant non-adherence than a coin toss (Meddings, Kerr, Heisler, & Hofer, 2012). Various factors have been individually associated with such medication-taking decisions including side effects, perceived drug effectiveness, and cost (Laba et al., 2012). Poor adherence to long-term therapies severely compromises the effectiveness of treatment making

this a critical issue in population health both from the perspective of quality of life and of health economics (World Health Organization, 2003). Poor medication adherence produces large downstream health care costs and policymakers contemplating changes in health policy should take note that reducing patients' out-of-pocket costs improves medication adherence (Viswanathan et al., 2012).

SUMMARY

The population of the United States is growing. According to the United States Census (2010) there are 203,891,983 (~66.0% of the total United States population of 308,745,538) million people living in the United States that are age 25+ years (United States Department of Commerce, 2011). With an increase in population comes an increasing number of people who may become vitamin B12 deficient. Tucker et al. (2000) suggests that at least 8.3% of the population (age 26+ years) may have blood levels of vitamin B12 that indicate deficiency. There are a number of ways to become deficient in vitamin B12 including, decreased consumption of vitamin B12, aging, medication use, and disease. There are two common ways to treat those with vitamin B12 deficiency, oral supplementation and intramuscular injection. With costs of supplementation varying greatly between oral and injectable vitamin B12 along with adherence levels of approximately 50% for those taking medications, every avenue of treatment should be investigated for effectiveness.

CHAPTER THREE

METHODOLOGY

INTRODUCTION

Vitamin B12 is a water-soluble vitamin that is required for several processes in the human body. These processes include DNA synthesis, red blood cell production, energy production, fatty acid synthesis, nervous system maintenance and the conversion of homocysteine into methionine (Butler et al., 2006). Typical absorption of orally consumed vitamin B12 is a complex multi-step process. A human can become deficient in vitamin B12 in different manners (i.e. surgically induced (Donadell et al., 2011), medication induced (World Health Organization, 2003), decreased consumption (Herbert, 1994) or decreased absorption (Goldman & Ausiello, 2004)) and can present with a myriad of clinical (Oh & Brown, 2003) and sub-clinical (Prevention, 2009) symptoms and/or conditions including fatigue, anemia, various psychoses, and various neurologic symptoms to name a few.

SELECTION OF SUBJECTS

This study was approved by the Institutional Review Board of the University of Arkansas which ensured safety of the subjects. The investigator recruited subjects by sending email notifications and word of mouth solicitation that asked for volunteers to take part in a 4-week randomized vitamin B12 study. These subjects were recruited from a large chiropractic clinic in Saint Louis, Missouri. A mass email was sent to the clinic database of email addresses of current and past clients of the clinic. From this email, participants contacted the researcher where basic information regarding the study was shared. Upon interest, the individuals came to the clinic where they were asked to read and sign the informed consent form and complete a health questionnaire. The questionnaire asked questions regarding their current and past medical

diagnoses, any medications the subjects may be taking and questions regarding their current level of activity.

INCLUSION CRITERIA

The inclusion criteria for this study were male and female subjects ages 18-49 years who either exercise or who do not exercise with an initial serum B12 level <1100 pg/mL. Exclusion criteria for this study were; answering yes to any of the questions on the health questionnaire with exception of the question regarding loss or change in sensation of the arms and/or legs. Subjects may have been included for the study if they have loss or change in sensation if the loss or change occurred due to trauma.

EXCLUSION CRITERIA

Any individual who met any one single or combination of the following criteria were excluded from the sample: pregnancy, previous diagnosis of a lung disorder (including asthma, emphysema, chronic or acute bronchitis, COPD), people who have received gastric bypass surgery or ileal resection, anyone diagnosed with neurologic or psychiatric symptoms/diseases including paresthesias, and psychotic conditions, diagnoses stage 4 kidney disease, inflammatory bowel disease including Chron's disease, ulcerative colitis and celiac disease, human immune-deficiency virus (HIV), vegetarians (non-meat eaters), those currently prescribed and taking metformin, a current serum B12 level >1100 pg/mL and those individuals currently supplementing with vitamin B12 (oral or intramuscular injection).

GROUP SELECTION

Participants who met the inclusion criteria for this study were placed into one four groups: non-exercise oral B12, non-exercise nebulized B12, exercise oral B12 or exercise nebulized B12. Individuals who were, at the beginning of the study, exercising at 65% of their

age predicted maximum heart rate at least three times per week, as reported on their health questionnaire, were placed randomly into either the exercise oral B12 or exercise nebulized B12 group. Participants who were non-exercisers were randomly placed into either the non-exercise oral B12 or non-exercise nebulized B12 group.

VITAMIN B12 ADMINISTRATION

Subjects randomly assigned into the oral supplementation group were given 28, 1 mg vitamin B12 supplements. They received instructions to take their vitamin B12 supplement upon waking each day for 28 consecutive days. Upon completion of the 28 consecutive days of oral supplementation the subjects had their blood drawn to re-test serum vitamin B12 levels.

Subjects randomly assigned into the nebulized vitamin B12 group made four weekly appointments to receive their nebulized vitamin B12 treatments. Each subject received one nebulized vitamin B12 treatment per visit. Each treatment occurred as follows:

1) The doctor assembled the mouthpiece for the nebulizer.
2) The doctor attached the mouthpiece hose to the nebulizer.
3) The doctor handed the mouthpiece to the subject.
4) The doctor drew 1 mg of cyanocobalamin into a 3 cc syringe.
5) The doctor injected the 1 mg of cyanocobalamin into the reservoir of the nebulizer.
6) The doctor ensured proper application and utilization of the mouthpiece by the subject.
7) The doctor turned on the nebulizer.
8) The doctor instructed the patient to breathe normally through the mouth until the vitamin B12 has disappeared from the reservoir.
9) The patient breathed normally as the nebulized vitamin B12 treatment was ongoing (approximately 15 minutes).

10) The doctor determined when each treatment was finished. This was done by observing that all of the vitamin B12 was gone from the reservoir.
11) The doctor turned off the nebulizer.
12) The doctor took the mouthpiece from the individual.
13) The doctor discarded the mouthpiece.
14) The doctor ensured the next treatment was scheduled for the subject.
15) The doctor dismissed the subject.
16) On the last day of treatment (4th treatment) an appointment was scheduled to have their serum B12 level re-tested within 48 hours of the final treatment.

VARIABLES

The primary outcome variable was post-test serum B12 levels. These levels were measured in all four groups: non-exercise oral B12, non-exercise nebulized B12, exercise oral B12 and exercise nebulized B12.

INSTRUMENTATION

Blood serum B12 levels were analyzed using Quest Laboratories blood serum immunoassay.

STASTISTICAL ANALYSIS

The subjects for this study were volunteer male and female adults, ages 18-49 years. These individuals had to have an initial serum B12 level <1100 pg/mL as measured by Quest Laboratories serum vitamin B12 immunoassay.

Statistical analysis of the data included demographic information including mean age, height, weight, and BMI for each of the four groups. A repeated-measures ANOVA was performed to test the interaction between groups. Main effects were performed for time.

Additionally a linear regression model was run to identify predictors of post-test vitamin B12 levels. The linear regression model was performed using backwards stepwise selection method (entry level, $p = .10$). In the final model only variables that met the designated alpha level were retained (stay level, $p = .05$). All statistics were computed using SPSS 20.0™ (SPSS, Inc., Chicago, IL). The overall statistical significance of this study was set at $p = .05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

INTRODUCTION

This study was a randomized trial investigating a different route of administration (nebulization) of vitamin B12 as a viable option for increasing serum B12 levels. There were 40 participants in the study. All participants were assigned to one of four groups before any of the data were collected. The four groups included a non-exercise oral B12 group, a non-exercise nebulized B12 group, an exercise oral B12 group, and an exercise nebulized B12 group. The participants in either the exercise or non-exercise oral B12 groups consumed a 1 milligram cobalamin (B12) supplement daily for 28 days. The participants in either the exercise or non-exercise nebulized B12 group were given four, once weekly, 1 milligram nebulized B12 treatments.

To answer the two null hypotheses a 2 X 2 repeated measures ANOVA was completed. The analysis assessed the difference in means of pre- to post-test serum B12 levels of nebulized and oral B12 supplementation between both exercises and non-exercisers.

RESULTS OF STATISTICAL ANALYSIS

There were 21 females and 19 males who participated in this study ($n = 40$). There were 21 participants who were assigned to the group oral B12 supplements and 19 who were assigned nebulized vitamin B12. Twenty-one participants were in the non-exercise group and 19 were assigned to the exercise group. Upon initial analysis, there were no differences for any variable between the two groups (Table 1).

Table 1. Demographic and Clinical Characteristics of the study sample. *n* = 40

Variable	Total	Oral	Nebulized	*p*
Participants	*n* = 40 mean(sd)	*n* = 21 mean(sd)	*n* = 19 mean(sd)	
Sex				0.199
Female	21	9	12	
Male	19	12	7	
Height, cm	172.80 (10.62)	175.13(10.26)	170.18(10.64)	0.142
Weight, kg	80.239(19.28)	77.54(16.88)	81.84(21.70)	0.361
BMI, mean(sd)	26.85(5.72)	25.24(4.14)	28.62(6.75)	0.062
Exercise Status				0.987
Non-Exerciser	21	11	10	
Exerciser	19	10	9	
Pre-B12 level, mean(sd)	559.65(190.96)	550.76(200.78)	569.47(184.46)	0.769
Post-B12 level, mean(sd)	753.11(320.62)	672.95(183.66)	847.41(416.84)	0.007

NULL HYPOTHESIS

The main objective of the investigation was to determine if a different delivery system (i.e. nebulization) of vitamin B12 could raise serum B12 levels compared to oral B12 levels in both an exercising and non-exercising population. For serum B12 levels there was no time by group interaction ($F[1,33] = 2.352$, $p = .135$) (Table 2). Similar results were seen for the time by exercise interaction ($F[1,33] = 3.013$, $p = .087$). There were also no significant differences for the time by group by exercise interaction ($F[1,33] = 1.472$, $p = .234$). Based on these finding the null hypothesis is accepted. There was no statistical difference in mean B12 levels between nebulized and oral groups. Also, there was no statistical difference in mean B12 levels between exercise and non-exercise groups. There were no differences noted for either the group (oral or nebulized) or exercise (exercise or non-exercise) with respect to serum B12 levels. The only significant outcome was time (pre- to post-test serum B12 levels) ($F[1,33]$, $p = <.05$).

Table 2. Tests of Within-Subjects Contrasts
Measure: B12

Source	Time	Type III Sum of Squares	df	Mean Square	*F*	*p*
Time	Linear	765487.384	1	765487.384	24.354	0.000
Time * Group	Linear	73917.244	1	73917.244	2.352	0.135
Time * Exercise	Linear	97529.989	1	97529.989	3.103	0.087
Time * Group * Exercise	Linear	46256.907	1	46256.907	1.472	0.234
Error(Time)	Linear	1037237.082	33	31431.427		

POST HOC ANALYSES

Since there were no statistical significances for oral vs nebulized or exercise vs non-exercise, a non-traditional approach to post hoc analyses was taken. A backwards stepwise linear regression was run to determine if any of the variables studied were significant predictors of post-test serum B12 levels.

The model summary showed an $R^2 = .323$ (Table 3). This indicated that pre-test B12 levels account for 32.3 % of the variability seen in the post-test B12 levels. The results of the ANOVA indicated that this regression model is a significant predictor of post-test B12 levels ($F[1,36] = 16.676$, $p = < .05$) (Table 4). Coefficient outputs and 95% confidence intervals were evaluated to examine the contributions of the variables. For pre-test B12 the coefficients were (β=.977) 95% CI [0.491, 1.462], $p = < .05$. These results indicate that as the pre-test B12 levels increase by one pg/mL we would expect to have post-B12 levels increase by 0.977 pg/mL, almost a one-to-one ratio.

INFLUENTIAL OUTLIERS

Both the repeated measures ANOVA and the regression model indicated that there were two outliers who were beyond two standard deviations from the mean. These two cases (case 37 and case 31) were investigated using casewise diagnostics to determine if either case had an effect on the statistical outcome of the study. The three tests that were performed were Mahalanobis distance, Cook's distance, and Cook's leverage (Table 5). In order for an outlier to impact the results of this study that outlier would have a Mahalanobis value greater than 15, a Cook's D value greater than 1, and a Cook's leverage value greater than 0.15. Neither outlier negatively impacted the results, therefore both outliers were left in the data.

Table 3. Regression Model Summary[f]

Model	*R*	*R* Square	Adjusted *R* Square	Std. Error of the Estimate
1	0.648[a]	0.420	0.326	263.1829
2	0.648[b]	0.420	0.347	259.0381
3	0.644[c]	0.414	0.361	256.2478
4	0.611[d]	0.373	0.336	261.1807
5	0.568[e]	0.323	0.303	267.6053

a. Predictors: (Constant), BMI, Sex, Exercise, Pre_B12, Group
b. Predictors: (Constant), Sex, Exercise, Pre_B12, Group
c. Predictors: (Constant), Exercise, Pre_B12, Group
d. Predictors: (Constant), Exercise, Pre_B12
e. Predictors: (Constant), Pre_B12
f. Dependent Variable: Post_B12

Table 4. ANOVA[a]

Model		Sum of Squares	*df*	Mean Square	*F*	*p*
1	Regression	1553418.541	5	310683.708	4.485	0.003[b]
	Residual	2147223.027	31	69265.259		
	Total	3700641.568	36			
2	Regression	1553418.475	4	388354.619	5.788	0.001[c]
	Residual	2147223.093	32	67100.722		
	Total	3700641.568	36			
3	Regression	1533765.493	3	511255.164	7.786	0.000[d]
	Residual	2166879.074	33	65662.911		
	Total	3700641.568	36			
4	Regression	1381320.198	2	690660.099	10.125	0.000[e]
	Residual	2319321.37	34	68215.334		
	Total	3700641.568	36			
5	Regression	1194201.018	1	1194201.018	16.676	0.000[f]
	Residual	2506440.55	35	71612.587		
	Total	3700641.568	36			

a. Dependent Variable: Post_B12
b. Predictors: (Constant), BMI, Sex, Exercise, Pre_B12, Group
c. Predictors: (Constant), Sex, Exercise, Pre_B12, Group
d. Predictors: (Constant), Exercise, Pre_B12, Group
e. Predictors: (Constant), Exercise, Pre_B12
f. Predictors: (Constant), Pre_B12

Table 5. Casewise Diagnostics (Summary of Outliers)

Case Number	Mahalanobis Distance (> 15)	Cook's Distance (> 1)	Cook's Leverage (> 0.15)
37	0.79560	0.27826	0.02210
31	0.00008	0.15773	0.00000

PEARSON'S CORRELATION

Pre-test B12 was the only significant predictor of post-test B12 levels, a Pearson's correlation was run to determine the linear correlation between the two variables (Table 6). The Pearson's correlation was $r = .568$ which was a moderate correlation.

RESEARCH HYPOTHESES

A clinical comparison of the pre-test and post-test means was performed to see the change in means for each of the four groups (Table 2). The means of every group increased. The oral exercise group's serum B12 level increased 20.6% from 572.6 pg/mL to 690.7 pg/mL. Cohen's effect size value ($d = .57$) (Table 7) suggested a moderate clinical significance. The oral non-exercise group's serum B12 level increased 23.8% from 530.9 pg/mL to 657.2 pg/mL. Cohen's effect size value ($d = .89$) suggested a high clinical significance. The nebulized exercise group's serum B12 level increased 28.5% from 582.9 pg/mL to 748.8 pg/mL. Cohen's effect size value ($d = .59$) suggested a moderate clinical significance. And lastly, the nebulized non-exercise group's serum B12 level increased 62% from 577.4 pg/mL to 935.1 pg/mL. Cohen's effect size value ($d = .92$) suggested a high clinical significance. Both research hypotheses (H1, H2) were accepted. Serum B12 levels increased in all four groups from pre-test to post-test. Also, due to the lack of significance found regarding the interaction between administration (oral vs nebulized) and exercise (exercise vs non-exercise) Cohen's effect sizes were calculated for the nebulized and oral groups. For the nebulized group, Cohen's effect size value ($d = .80$) suggested a large clinical significance. For the oral group, Cohen's effect size value ($d = .62$) suggested a moderate clinical significance.

Table 6. Pearson Correlation

		Pre-Test B12	Post-test B12
Pre-test B12	Pearson Correlation	1	0.568*
	Sig. (2 tailed)		0.000
	N	40	37
Post-test B12	Pearson Correlation	0.568*	1
	Sig. (2 tailed)	0.000	
	N	37	40

* Correlation is significant at the 0.01 level (2-tailed)

Table 7. Effect Sizes

Group	Cohen's *d* value	Clinical Effect
Oral Exercise	.57	medium
Oral Non-Exercise	.89	large
Nebulized Exercise	.59	medium
Nebulized Non-Exercise	.92	large
All Oral	.62	medium
All Nebulized	.80	large

DISCUSSION

Vitamin B12 is important for various processes in the human body including DNA synthesis, red blood cell production, energy production, fatty acid synthesis, and for the conversion of homocysteine into methionine. The typical absorption of vitamin B12 is dependent upon several steps. Vitamin B12 deficiency is becoming a more prevalent problem in our society. In the United States, more than 20% of the elderly have serum vitamin B12 concentrations that indicate depletion, and an additional 6% have deficiency (L. Allen, Rosenberg, Oakley, & Omenn, 2010). As vitamin B12 status becomes a health issue, there are then implications for out of pocket health care costs.

Healthcare costs are rising and are a real concern to many. Not only are people dealing with increased costs of medical services and procedures, they are also dealing with increased costs of having adequate health insurance coverage in the event of serious illness. Examining and exploring the cost of utilizing nebulized vitamin B12 as an at home treatment rather than an in clinic service over time could prove meaningful in the larger scope of healthcare costs. If weekly nebulization of any vitamin, mineral, amino acid, or medication is as effective at increasing the level of that substance in the body as taking daily pills, the significance of this study may have lasting ramifications on healthcare by providing more cost effective treatment options for both doctors and patients with regards to many different types of dietary supplements or medications

When comparing the results of this study to a similar vitamin B12 study, the results are trending toward significance. In the study that compared oral B12 supplementation to injectable B12 supplementation, the authors found a serum B12 increase of 242% in the injectable B12 group (Kuzminski et al., 1998). That study investigated the impact of 9 injections of vitamin

B12 over the course of eight weeks and the participants of the study had a starting mean serum B12 level of 95 pg/mL, which is considered vitamin B12 deficient. By comparison, this study, did not allow for any participant to be considered vitamin B12 deficient and the participants only nebulized B12 four times over the course of four weeks, yet the participants who nebulized vitamin B12 increased their serum B12 levels by an average of 28.5% for those who exercised and 62% for those who did not exercise.

Other substances have been shown to be useful in nebulized form. Nebulized glutathione has been shown to be an effective treatment for a variety of pulmonary diseases and/or respiratory-related conditions with very serious and difficult-to-treat diseases (e.g., cystic fibrosis, idiopathic pulmonary fibrosis) (Prousky, 2008). Blitz et al. (2005) concluded that nebulized magnesium sulfate appears to be a safe and effective treatment to administer to patients experiencing asthma exacerbations and he indicated that it has been demonstrated that therapy with magnesium sulfate and beta-2 agonists improved lung function when compared to therapy utilizing beta-2 agonists alone and that treatment with nebulized magnesium sulfate should be considered as an addition to inhaled beta-2 agonist therapy in patients experiencing asthma exacerbations.

Although no statistical significance was observed in this study, from a clinical standpoint, the results of the study are important. The effect sizes observed from the Cohen's *d* calculations bear discussion. If only looking at the participants who nebulized vitamin B12 versus those who took oral supplementation, without considering exercise status, the effect size for nebulizing vitamin B12 ($d = .80$) suggests that nebulizing vitamin B12 has a large clinical effect. An effect size of this magnitude may encourage a physician to suggest nebulization of vitamin B12 as a viable option to increasing a patient's serum B12 level.

When considering a patient who may need vitamin B12 supplementation, it is important for the ordering physician to know the willingness of each patient to follow-thru with treatment. As previously mentioned, people do not adhere to taking medication orally, with approximately 50% abandoning the treatments (Fuster, 2012; Laba et al., 2012). Also it has been noted that people do not like to have vitamin B12 injections due to several factors including pain and cost of treatment (Kwong et al., 2005). These reasons could have a large impact on the willingness to adhere to treatment. It should also be noted that most elderly persons who need to supplement with vitamin B12 are already taking several medications and they are possibly on a fixed income. The current methods of increasing serum B12 levels are to take more pills or to pay for expensive shots. By offering a different mode of administration of vitamin B12, a doctor would be taking into consideration the desire of the patient to not have another pill to take, as well as the possibility of ones fear toward needles coupled with the increased cost of injectables.

CHAPTER FIVE

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

SUMMARY

The purpose of this study was to evaluate the effect of nebulized vitamin B12 on serum B12 levels. Participants, ages 18-46, volunteered to participate in a vitamin B12 supplement intervention designed to study the effects of supplement type (oral or nebulized) and activity (exercise or non-exercise) on serum B12 levels. The participants of the study were assigned to one of four groups, non-exercise nebulized B12, exercise nebulized B12, non-exercise oral B12, and exercise oral B12. Those individuals in the oral B12 groups consumed 1 mg of vitamin B12 daily by mouth for 28 consecutive days. Those individuals in the nebulized B12 group were given a nebulized B12 treatment consisting of 1 mg of B12 one time per week for four consecutive weeks.

The results of this study found no statistically significant differences in mean serum B12 levels for any interaction (time by group, time by exercise, time by group by exercise) from the repeated measures ANOVA. Although, no statistical significance was observed, clinically, the nebulized vitamin B12 groups did increase their serum B12 levels more than the participants taking oral supplementation. Therefore, the results indicated that nebulized B12 can elevate serum B12 levels.

CONCLUSION

Based upon the results of the study it can be concluded that nebulized B12 is at least as effective at raising serum B12 levels as oral vitamin B12 supplementation. The results of the study are similar to those involving injectable vitamin B12 as well as those involving other substances (i.e., glutathione, magnesium) in that the results indicated that nebulizing vitamin B12

does in fact increase serum B12 levels. It is no secret that inhaling substances can increase the level of said substances in the body. For example, cigarette smoking increases nicotine levels in the blood, as does inhaling even more harmful compounds such carbon monoxide or radon. These substances get into the blood via simple diffusion in the alveolar sac. As the concentration of the substance increases in the alveoli, the red blood cells travel near and release carbon dioxide and in turn will pick up whatever substance happens to be there, in the case of this study, it was vitamin B12, and instantaneously the vitamin B12 is being transported in the blood. It is generally understood that serum B12 levels would increase by nebulizing vitamin B12, the interesting finding is that it seems to be as effective as oral supplementation and injections of vitamin B12 at increasing serum B12 levels.

RECOMMENDATIONS

The scope of this study did not investigate the effect of nebulizing vitamin B12 on serum B12 levels for individuals with impaired absorption abilities of vitamin B12 due to such factors as atrophic gastritis, gastric bypass surgery, taking metformin, or taking proton pump inhibitors/H2 receptor antagonists. However the study does suggest that nebulizing vitamin B12 may be a viable treatment option to assist in elevating serum B12 levels in these individuals. All factors mentioned above involve impaired (gastric bypass, atrophic gastritis) or impairing (medications) the absorption process of vitamin B12. Nebulization skips the complex multi-step absorption process in the gastrointestinal system and delivers the vitamin directly into the blood stream via the alveolar exchange in the lungs. This study shows that this method of delivery can and does raise serum B12 levels similar to or greater than oral B12 supplementation.

Some other alternative should be available to ensure that patients in need of vitamin B12 can get the treatments they need without the risk of stopping their treatment or the pain and other

risks associated with injectable forms of vitamin B12. Nebulized vitamin B12 is painless, quick, and based upon this study increases, albeit not statistically significantly, serum B12 levels greater than oral B12 supplementation.

For future studies it is recommended to perform a similar study looking at a specific population such as the elderly, vegetarians, or individuals taking medications that impair with the absorption of vitamin B12. It may be beneficial to determine if similar increases in serum B12 levels is expected in these populations. Another recommendation would be to investigate exercising individuals and define "exercisers" differently such as aerobic versus resistance training. One may also investigate longer treatment times to determine if treatment time has an impact on serum B12 levels. And lastly, it may be important to examine the overall cost of utilizing nebulization as a delivery system, such as the manufacturing costs as the delivery method becomes more readily available.

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APPENDIX A

Informed Consent

A Comparison of Nebulized Vitamin B12 Versus Oral Vitamin B12 Supplementation

Consent to Participate in a Research Study
Principal Researcher: Terry Williams Jr.
Faculty Advisor: Ro DiBrezzo, PhD

INVITATION TO PARTICIPATE
You are invited to participate in a research study about vitamin B12. You are being asked to participate in this study because you may benefit from an alternative administration route for vitamin B12.

WHAT YOU SHOULD KNOW ABOUT THE RESEARCH STUDY

Who is the Principal Researcher?
Terry Williams Jr.
5018 W. Homespun Dr.
Fayetteville, AR 72704
479-200-3134

Who is the Faculty Advisor?
Ro DiBrezzo, Ph.D.
422 Administration Building
Fayetteville, AR 72701
479-575-2151

What is the purpose of this research study?
The purpose of this study is to evaluate if a different route of administration, i.e. nebulization, is a viable option for increasing serum levels of vitamin B12.

Who will participate in this study?
The participants of this study will be male and female subjects ages 18-49 years who either exercise (defined as exercising at 65% of age predicted maximum heart rate at least 3 times per week) or who do not exercise (defined as individual who do not exercise at 65% of their age predicted maximum heart rate at least 3 times per week) with an initial serum B12 level <1100 pg/mL

What am I being asked to do?
Your participation will require the following:

1. You will be required to fill out a health questionnaire.
2. You will be required to have your blood drawn at Quest Laboratories to have your serum vitamin B12 level measured
3. If included in the study, you will be placed randomly into either the nebulized or oral vitamin B12 groups.
4. If placed in one of the oral B12 groups, you will be required to take 1 vitamin B12 supplement pill per day for 28 consecutive days.

5. If placed in one of the nebulized B12 groups, you will be required to visit the office one time per week for four consecutive weeks to have a nebulized vitamin B12 treatment administered to you.
6. Upon completion of the supplementation portion of the study, you will be required to have your blood drawn again at Quest Laboratories to have your serum B12 level measured.

What are the possible risks or discomforts?
Risks associated with having your blood drawn include slight pain, bleeding, bruising and swelling. There are no known risks associated with vitamin B12 supplementation.

What are the possible benefits of this study?
The possible benefit of this study is finding a different administration route for vitamin B12.

How long will the study last?
The study will require approximately 15 minutes to complete a health questionnaire. It will also require two visits to Quest Laboratories to have your blood drawn. If you are a participant in one of the nebulized B12 groups, you will be required to visit the office once per week for approximately 15-30 minutes per visit.

Will I receive compensation for my time and inconvenience if I choose to participate in this study?
You will not receive compensation for your time and inconvenience should you choose to participate in this study.

Will I have to pay for anything?
No, there will be no associated cost for you to participate in this study.

What are the options if I do not want to be in the study?
If you do not want to be in this study, you may refuse to participate. Also, you may refuse to participate at any time during the study. Your job, your grade, and/or your relationship with the University of Arkansas will not be affected in any way if you refuse to participate.

How will my confidentiality be protected?
All information will be kept confidential to the extent allowed by applicable State and Federal law.
All records will be locked in a secure area.

Will I know the results of the study?
At the conclusion of the study you will have the right to request feedback about the results. You may contact the faculty advisor, Dr. Ro DiBrezzo at 479-575-2151 or Principal Researcher, Terry Williams, Jr at 479-200-3134. You will receive a copy of this form for your files.

What do I do if I have questions about the research study?

You have the right to contact the Principal Researcher or Faculty Advisor as listed below for any concerns that you may have.

Terry Williams Jr.
XXXXXXXXXXXXXX
XXXXXXXXXXXXX
XXXXXXXXXXXX

Ro DiBrezzo, Ph.D.
422 Administration Building
Fayetteville, AR 72701
479-575-2151

You may also contact the University of Arkansas Research Compliance office listed below if you have questions about your rights as a participant, or to discuss any concerns about, or problems with the research.

Ro Windwalker, CIP
Institutional Review Board Coordinator
Research Compliance
University of Arkansas
210 Administration
Fayetteville, AR 72701-1201
479-575-2208
irb@uark.edu

I have read the above statement and have been able to ask questions and express concerns, which have been satisfactorily responded to by the investigator. I understand the purpose of the study as well as the potential benefits and risks that are involved. I understand that participation is voluntary. I understand that significant new findings developed during this research will be shared with the participant. I understand that no rights have been waived by signing the consent form. I have been given a copy of the consent form.

Printed Name: _____

Signature: _____

Date: _____

Phone _____

APPENDIX B

Health Questionnaire

Health Questionnaire

Name: _____

Sex: Male Female

Age: _____

Date of birth: _____

Are you currently taking vitamin B12 supplements either oral or injections? Yes No

Are you currently a vegetarian (does not eat animal products of any kind)? Yes No

Are you currently pregnant? Yes No

Have you ever received gastric bypass surgery? Yes No

Have you ever had ileal resection surgery? Yes No

Have you ever been diagnosed with any mental disorder including but not limited to: dementia, schizophrenia, Alzheimer's, or depression? Yes No

Have you ever experienced a lack of sensation or feeling in your arms or legs? Yes No

If yes, please explain: _____

Have you ever been diagnosed with kidney disease? Yes No

Have you ever been diagnosed with bowel disease including inflammatory bowel disease, Chron's disease, ulcerative colitis, atrophic gastritis or celiac disease? Yes No

Are you currently taking metformin? Yes No

Have you ever been diagnosed with HIV/AIDS? Yes No

To Be Completed by the Investigator:

Do you currently exercise? Yes No

If yes, do you exercise at a target heart rate of 65% of your age predicted maximal heart rate on three or more days per week?

Yes No

Calculation for target heart rate of 65% of age predicted maximal heart rate.

Heart Rate: 220 - _____(age) = _____ (age predicted max heart rate)

.65 x _____(age predicted max heart rate) = _____(target heart rate)

APPENDIX C

Institutional Review Board Approval

May 6, 2013

MEMORANDUM

TO:	Terry Williams, Jr. Ro DiBrezzo
FROM:	Ro Windwalker IRB Coordinator
RE:	New Protocol Approval
IRB Protocol #:	13-04-665
Protocol Title:	*The Effects of Nebulized Vitamin B12 on Serum B12 Levels: A Comparison of Nebulized Vitamin B12 versus Oral B12 Supplementation in an Exercise and Non-Exercise Population*
Review Type:	☐ EXEMPT ☒ EXPEDITED ☐ FULL IRB
Approved Project Period:	Start Date: 05/03/2013 Expiration Date: 04/28/2014

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://vpred.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 50 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.