Vitamin B12 Deficiency in Individuals with Diabetic Neuropathy

By
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Date approved: November 21, 2016

Abstract

Vitamin B12 is a water soluble vitamin known for its many deficiency symptoms. New studies on demographics and serum vitamin B12 levels conflict with past results. The purpose of this study was to evaluate the relationships among weight status, serum vitamin B12 levels, and vitamin B12 dietary intake in individuals with Type 2 diabetes and diabetic neuropathy.

A total of 30 participants were recruited from the University of Kansas Medical Center and the University of Utah. In a randomized exercise intervention trial (Activity for Diabetic Polyneuropathy: ADAPT Study) height, weight, Body Mass Index, medication, supplement use, age, serum vitamin B12 values and dietary intake via 3 day food records were collected at baseline visits. The 3 day food records were entered into a nutrition software program (NDSR) to determine sources and amount of vitamin B12 consumed for a 3 day average. Descriptive statistics were used to characterize the participants. Differences between metformin vs. non-metformin users were compared using ANOVA. Regression analysis was used to evaluate the relationship between serum vitamin B12 and the following variables: weight, age, vitamin B12 supplement use and oral B12 consumption. A pairwise correlation matrix linked each independent variable in a set point in time.

The mean total vitamin B12 consumption was 4.3 µg per day. The use of the drug metformin was not found to be statistically significantly related to serum B12 levels. No relationships were found between serum vitamin B12 and age, BMI, or obesity status. The use of vitamin B12 supplements was positively related to serum vitamin B12 levels, independent of dosage ($p = .031$).

These findings are encouraging for those who are at risk for vitamin B12 deficiency. The study also does not find a negative correlation between age and serum B12 levels, which could indicate that screening for deficiency should start at a younger age. Future studies should look at age and its relationship to serum B12 levels.

Acknowledgement

I cannot express enough thanks to my committee, and statistical team for their continued support and encouragement. I owe my gratitude to all these people for their time, experience and mentoring throughout my journey.

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Angela Cusimano

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Chapter 1: Introduction

According to the Centers for Disease Control and Prevention (CDC), 29 million people in the United States have diabetes mellitus (1). The World Health Organization (WHO) has estimated that 90% of people with Type 2 diabetes are overweight or obese (2). Of the 27.5 million with type 2 diabetes, 60-70% will develop peripheral neuropathy (DPN) (3) and 22% are found to be vitamin B12 deficient (4). Signs and symptoms of both DPN and vitamin B12 deficiency are similar and therefore patients undergoing testing for DPN must first eliminate vitamin B12 deficiency as the problem. In addition, patients consuming the diabetes medication, metformin, increase their risk of vitamin B12 deficiency by 74% (5) , compared with patients taking insulin and other forms of biguanides. Other factors can also attribute to low vitamin B12 levels, including age, diet, malabsorption and other medication usage (6). Many studies have focused on age and the use of metformin as the source of vitamin B12 deficiency.

In 1994, the Framingham study looked at vitamin B12 deficiency in an elderly population (≥65 years) (7). They found 40% of individuals 65 years and older were deficient while 17.9% of subjects 22-63 years old were deficient (7). Couderc et al found that 57.6% of his participants (≥ 64 years) had food cobalamin malabsorption while 12% had nutritional cobalamin deficiency, due to inadequate intake of oral vitamin B12 (8). Solomon et al concluded that serum B12 in individuals >70 years old was significantly lower than individuals 60-69 years old (9). In contrast, El-Khateeb in 2014 found that younger adults in Jordan (19-30 years old) had the highest rates of vitamin B12 deficiency. The study cites multiple other studies with the same conclusion (10). Yajnik's cross-sectional study found over 67% of men ages 30-50 in rural India were vitamin B12 deficient (11). Similar inconsistencies can be found in the usage of metformin and other causes of vitamin B12 deficiency.

Metformin is the number one recommended drug for individuals diagnosed with pre-diabetes and diabetes (12-14). While most studies conclude that patients taking metformin have a 10-30% risk of developing vitamin B12 deficiency, there is not consistency in the timeline (4, 5, 14). Bell et al stated that it takes 12 to 15 years of inadequate vitamin B12 intake in metformin users for serum B12 to reflect depleted stores (12). Bauman et al's clinical trial, however, found a decrease in serum B12 after only 3 months of metformin use (13). The National Health and Examination Survey, 1999-2006 cross sectional study, found that duration of metformin use increased the risk of vitamin B12 deficiency, but the increased risk was not statistically significant (15). Patients taking the medication for <1 year had a 4.1% risk, while there was a 8.1% risk in subjects taking metformin for >10 years (15). Pflipsen et al conducted a cross-sectional study on outpatients with Type 2 diabetes. Their study revealed that metformin increased the risk of vitamin B12 deficiency, but dosage was not a factor. They specified chronic metformin use increased the risk of deficiency, but did not specify the timeframe the authors considered chronic. A clinical trial by Singh et al found vitamin B12 deficiency with metformin only after 1 year of use (28.5%), and that individuals taking metformin had a statistically significant increase in peripheral neuropathy (48.8%) (5).

Metformin use, age and other medications have been studied, but there are still gaps in the literature. Few studies have evaluated body weight and its relationship to vitamin B12 deficiency. Only two studies related vitamin B12 deficiency to obesity (10, 16). A population based study found that 30.5% of normal and overweight older adults were vitamin B12 deficient, while 34.4% of obese older adults were deficient. A prospective descriptive study showed obese children had an average of 130pg/mL lower serum B12 when compared to normal weight children. Ten percent of obese children had low serum B12 while, 2.2% of normal weight

children had low levels (16). Neither study looked at obesity as an independent variable of vitamin B12 deficiency.

The success of vitamin B12 supplementation regarding deficiency and DPN is inconsistent and variable (8). The National Health and Nutrition Examination Survey (NHANES) found no association between a lower risk of vitamin B12 deficiency and supplementation in individuals taking metformin. Adults taking up to 6µg of vitamin B12 had a 14.1% risk of becoming deficient, while people taking >6µg of vitamin B12 had a 1.8% risk of developing deficiency (15). The Framingham Offspring prospective Cohort study of Healthy Adults, 26 years of age and older, looked at fortified foods, and animal sources of vitamin B12. (17). The study found that people who consumed fortified cereal 4 times a week or more, had a 14% risk of becoming deficient. People who did not consume fortified cereals had a 23% risk of becoming deficient. The study also concluded that fortified foods might be better absorbed than meats and other protein sources.

Statement of Purpose

The purpose of this proposed thesis was to further research the correlations between weight status, serum vitamin B12 levels, and vitamin B12 dietary intake in individuals with Type 2 diabetes and diabetic neuropathy

Research Questions

a. What is the average daily intake (µg) of vitamin B12 in adults with diabetic peripheral neuropathy
b. Is there a difference in serum vitamin B12 levels between metformin vs non-metformin users?
c. Is there a relationship between vitamin B12 consumption and serum B12 levels?
d. Is there a relationship between Body Mass Index and serum B12 levels?
e. Is there a relationship between age and serum B12 levels?

Chapter 2: Review of Literature

Introduction:

More than 29 million Americans have been diagnosed with diabetes mellitus (DM) (18). From this group, 60-70% have some form of diabetic neuropathy (19). Diabetes mellitus is now the 7th leading cause of death in the United States. Complications related to DM include hypertension, stroke, heart attacks, blindness, kidney disease, amputations, cobalamin deficiency and death (1).

Cobalamin (Vitamin B12) deficiency is found in 20% of people over the age of 65 (6, 8). Certain factors such as diet, age and medications can increase the risk of developing a deficiency (20). The purpose of this Literature Review is to review the relationships between weight status, serum B12 levels and vitamin B12 dietary intake in adults with diabetic neuropathy.

Diabetic Neuropathy:

Types of Diabetes Mellitus

There are two main types of DM; Type 1 and Type 2 (21). Type 2 Diabetes (T2DM) is when a hormone called insulin, produced by the pancreas, does not work properly. The insulin is not able to pull glucose from the blood for cells to use as fuel (22). The pancreas then tries to overproduce insulin to rectify the problem. The body becomes insulin resistant, which is when insulin no longer works properly, leading to hyperglycemia (23). Overweight and obesity is seen in 60-90% of individuals with T2DM (21). Other risk factors include decreased physical activity and genetics (6).

High blood glucose levels produce many co-morbidities. Consistent hyperglycemia leads to plaque formation in the arteries. This narrows the vessels and can lead to a stroke or heart attack. Kidney damage occurs when the kidneys are required to filter out the extra glucose in the blood (23). After time, the kidneys fatigue, leading to failure, potentially requiring hemodialysis.

Diabetic neuropathy is the damage of blood vessels that carry oxygen to the nerves of organs, the gastrointestinal tract, extremities and the cardiovascular system (19). Neuropathy is found in 60-70% of individuals with T2DM (19). Chronic high blood glucose causes ischemia, the narrowing of the vessels, preventing oxygen and nutrients to travel throughout the body (23). Loss of sensation to extremities often leads to injury. When a patient with diabetic neuropathy has a wound, blood, nutrients, and oxygen are not able to heal the foot or hands effectively. The wound, left untreated, can lead to a diabetic foot ulcer. Eighty-four percent of diabetic foot ulcers lead to lower-leg amputations (24).

There are two main forms of diabetic neuropathy, chronic sensorimotor and autonomic neuropathy (2-4, 10). Chronic sensorimotor diabetic peripheral neuropathy is found in about 50% of patients with neuropathy (25). Occurring in the lower limbs, hands and feet; symptoms include, warm or cold feet, burning pain, stabbing feelings, tingling sensation, numbing, or deep aching pain in the localized area (19, 25). Autonomic neuropathy (AN) is related to the sympathetic and parasympathetic nervous systems. Autonomic neuropathy can affect the nerves related to the gastrointestinal tract, cardiovascular system, lungs and eyes (19). The main side effect of AN in the gastrointestinal tract is gastroparesis. In this condition the stomach delays gastric emptying, which can cause constipation, abdominal pain, and heart burn. Other side effects of AN can include low blood pressure, blindness and trouble breathing. (3, 4)

Medications for Type 2 Diabetes:

The largest non-insulin medication used for diabetes and pre-diabetes is metformin. It is prescribed to over 120 million people in the world, around 1.6% of the population (26). Metformin acts as an anti-hyperglycemic agent, preventing high blood glucose levels. The medication decreases hepatic glucose production by preventing the liver from producing glucose from non-carbohydrate sources. This lowers blood glucose without a risk of hypoglycemia or weight gain (26). Side effects of the drug are lactic acidosis, abdominal pain, diarrhea and vitamin B12 deficiency (27). The use of metformin lowers vitamin B12 levels by 22% compared to individuals with diabetes who were not taking metformin (5).

Vitamin B12

Vitamin B12, also known as Cobalamin, is a water-soluble vitamin, a cofactor for two main enzyme reactions, and a feature in myelin production (6). The first enzyme reaction vitamin B12 is involved in is the conversion of methylmalonyl CoA to succinyl CoA. Succinyl CoA is an important intermediate of the Citric Acid Cycle, which produces ATP. The second vitamin B12 enzyme reaction is the conversion of homocysteine to methionine. Vitamin B12 binds to methionine synthase and the following process creates methylcobalamin and 5-methyl tetrahydrofolate (THF). Inadequate vitamin B12 can lead to higher serum homocysteine and THF levels. THF is important for many coenzymes, while high levels of serum homocysteine can lead to cardiovascular problems. Once methionine is created, it is then converted to S-adenosylmethionine (SAMe) which is a methyl donor. SAMe is needed for the protein myelin, which produces the myelin sheath.

The term vitamin B12 and cobalamin are interchangeable. The term cyanocobalamin refers to the synthetic form of vitamin B12 that is taken in supplement form. Cyanocobalamin must first be converted to an active form before being absorbed (23).

Absorption:

Cobalamin absorption starts in the mouth. Saliva, along with food containing vitamin B12 travel to the stomach. The stomach releases hydrochloric acid (HCl), pepsinogen and intrinsic factor. Hydrochloric acid then activates pepsinogen to pepsin, a main digestive enzyme (23). An acidic environment is important to activate the stomach enzymes. Pepsin and HCl separate vitamin B12 from its food source. The free vitamin B12 then binds with transcobalamin 1 (TCI) from saliva in the stomach. The TCI-Vitamin B12 complex travels to the duodenum where vitamin B12 is separated from TCI and attaches to intrinsic factor, which has traveled from the stomach. The vitamin B12 intrinsic factor complex travels to the ileum where intrinsic factor receptors allow vitamin B12 absorption into the plasma. The intrinsic factor receptors that facilitate vitamin B12 into the blood are calcium dependent (13). Calcium supplementation used to prevent vitamin B12 deficiency will be discussed in further detail. Once across the ileum surface membrane, vitamin B12 attaches to either TCI or TCII where it is released into the portal vein for transport to peripheral tissue (23).

Function:

Vitamin B12 is important for DNA, RNA and red blood cell production. Along with being required for two enzymatic reactions, it also plays an important role in lipid synthesis, which produces the myelin sheath. The myelin sheath wraps around nerves and protects the axon and the nerves from being exposed (28). The conversion of homocysteine back to methionine requires the vitamin B12 enzyme. Vitamin B12 deficiency leads to excessive amounts of serum

homocysteine (23). Malabsorption or deficiency of vitamin B12 can lead to megaloblastic anemia, neuropathy, folate deficiency and cardiovascular disease due to chronic high serum homocysteine levels (29).

Vitamin B12 deficiency:

Vitamin B12 deficiency is most common in the elderly, vegans, individuals who have had any gastrointestinal surgeries and people taking certain medications: proton pump inhibitors (PPI) and metformin. Vitamin B12 deficiency affects >20% of people over the age of 65 (6). This deficiency is frequently undiagnosed because of non-distinguishable signs and symptoms. The two main causes of vitamin B12 deficiency in the elderly are malabsorption and pernicious anemia (6). Gastric atrophy, the degeneration or wasting away of cells, occurs in 40% of people over the age of 80 (1). This atrophy prevents free vitamin B12 from binding to intrinsic factor where it is then absorbed into the blood stream. (8). Bacterial overgrowth, seen with antibiotic use, can cause inflammation of the small intestine, which can prevent vitamin B12 absorption (1).

Pernicious anemia is the second main cause of vitamin B12 deficiency. Twenty to forty percent of elderly have vitamin B12 deficiency due to pernicious anemia (6). This autoimmune disease destroys the cells in the stomach that produce intrinsic factor. In addition to pernicious anemia, those taking proton pump inhibitors are also at risk of vitamin B12 deficiency. Proton pump inhibitors, decrease the acidity of the stomach, thus reducing intrinsic factor needed for absorption. The consumption of alcohol for more than 2 weeks consistently is also seen to increase vitamin B12 malabsorption (2).

Signs and symptoms

Signs and symptoms of vitamin B12 deficiency can include symptoms of megaloblastic anemia, fatigue, weakness, abdominal pain and gastroparesis, along with neurological conditions such as memory loss, mood changes and neuropathy of the hands and feet (1). Excessive serum homocysteine has been linked to increased risk of coronary heart disease and stroke (30). Homocysteine can decrease vasomotor function of the endothelia and can increase the oxidative degradation of lipids (30).

Vitamin B12 food sources:

Vitamin B12 is largely found in meats, fish, shellfish, and dairy products (1). The current RDA for vitamin B12 ages 12+ is 2.4 µg, while there is no RDA for the elderly (2). The NHANES 1999-2000 concluded that the average daily consumption of vitamin B12 in the United States was 3.4 µg (30). The US Department of Agriculture (USDA) has compiled a database of food sources with their concentrations of vitamin B12 (e.g. fortified breakfast cereals contain 6 µg per serving, a cheeseburger with two patties contains 2.1 µg, and 8 ounces of yogurt contains 1.1µg (1). Other sources of vitamin B12 include supplementation.

Laboratory status:

The laboratory status of serum cobalamin is the main indicator of vitamin B12 deficiency. While the precise laboratory value for deficiency may vary slightly between laboratories, most major studies use ~150 pmol/L and under, as their diagnostic criteria (31). The serum B12 measures cobalamin bound to TCI and TCII. TCI is the only B12 bound protein used for metabolic demands (31). Only evaluating TCI when determining appropriate serum B12 levels could give false high values. Calculating serum B12 levels must be evaluated on an individual bases. Serum homocysteine and holotranscobalamine are two other indicators of

deficiency. Excess serum homocysteine of >20 µmol/L, indicates homocysteine is not being converted back to methionine via vitamin B12 enzymes. Holotranscobalamin is the active form of cobalamin taken into the cells. A level of <35 pmol/L indicates a deficiency of the active form of vitamin B12.

Metformin and vitamin B12 deficiency:

The majority of recent studies conducted on vitamin B12 deficiency are related to the use of the medication metformin. Three were cross-sectional studies of individuals with diabetes taking versus not taking metformin (4, 5, 15). All studies were under 12 months in length, and measured deficiency as serum B12 <100 pg/mL or <150 pg/mL. One study by Pflipsen et al, in the United States, found 22% of their study population to be vitamin B12 deficient, and patients taking metformin had a higher statistical risk of developing vitamin B12 deficiency (4). They did not find a statistical significance between the dosage of metformin and vitamin B12 deficiency. The second study, by Singh et al in India, found that 28.5% of their patients taking metformin were vitamin B12 deficient; while only 4.7% not taking metformin were deficient (5). NHANES 1999-2006 discovered 5.8% of their population with diabetes taking metformin were vitamin B12 deficient, while 3.3% of those not taking metformin were deficient (15). In conclusion, the studies showed metformin was associated with lower serum B12 levels.

Two clinical trials looked at the use of metformin in participants with diabetes compared to a placebo (13, 14). Bauman et al completed a 6-month study with participants consuming 850 mg of metformin or a placebo pill twice a day. They found a statistically significant difference in serum B12 levels between the metformin and placebo groups after 3 months The metformin therapy subjects had an average of a 30% decrease in serum B12 more than the control group

(13). Jager et al conducted a 4.3 yearlong study where participants took 850 mg metformin or a placebo 3 times a day. The long-term study found a 19% decrease in vitamin B12 levels compared to the placebo group. The metformin group also had a 7% higher risk of developing vitamin B12 deficiency (13). Overall, both studies support the theory metformin increases the risk vitamin B12 deficiency.

Vitamin B12 deficiency not related to metformin

Although the use of metformin is known to cause lower levels of vitamin B12, other factors such as age, other medication use and diet can also lower vitamin B12 levels. Seven studies found that the risk of deficiency increases with age (6, 8, 14, 15, 20, 32-34). Cutoff age range varied from ≥ 65 (34) to ≥ 30 years old (14). The average percent of vitamin B12 deficiency in people over the age of 65 with or without diabetes ranged from 12% up to 64% (6, 33). Couderc et al conducted a retrospective study in France of older adults in an acute hospital setting and found that 57.6% of patients 65 years and older had food-cobalamin malabsorption (8). However in 2014 El-Khateeb, a population based study in the country Jordan, found adults under the age of 65 had a higher risk of developing deficiency than adults 65 years or older (10). In conclusion, the age of vitamin B12 deficiency was variable and further studies would be beneficial.

Medication Use

Proton pump inhibitors (PPI) are a type of medication used to relieve the symptoms of heartburn and acid reflux. The purpose of PPI's is to decrease the acidity of the stomach, preventing acidic stomach contents from flowing into the esophagus. Presse et al, a study evaluating hospital discharges, looked at the rate of vitamin B12 deficiency and PPI use. Forty

two percent of the patients were taking some kind of antacid while 41.3% of discharges were vitamin B12 deficient (35). The study concluded that the use of PPI's increased the risk of developing vitamin B12 deficiency.

Vegetarians

Meats have one of the highest concentrations of vitamin B12 in relation to food sources (1). As a result, vegetarians and vegans are both groups with a high risk of vitamin B12 deficiency (5). Kumar found that vegans had a higher rate of vitamin B12 deficiency that was statistically significant compared to non-vegans. Their data were not dependent on the use of metformin (21).

Neuropathy related to vitamin B12 deficiency

Peripheral neuropathy is a well known symptom of vitamin B12 deficiency (5, 9, 12, 36). A retrospective study of patients with vitamin B12 deficiency from a primary care practice found that 62% of their patients with diabetes had a form of neuropathy (9). A long term cross sectional study also found that 75% of patients with diabetes taking metformin had neuropathy, while 46.5% of those not taking metformin had a form of neuropathy (5). The authors concluded that metformin had a significant negative correlation on risk of neuropathy (16). Another cross-sectional longitudinal study concluded that 60% of those with insulin-dependent diabetes had a form of neuropathy and 59% of non-insulin dependent patients had neuropathy. Symptoms of neuropathy only occurred in 20% of all of their participants with neuropathy (36). Overall 60-70% of individuals with diabetes, not dependent on medication or insulin use, will have a risk of developing neuropathy.

Timeframe:

The signs and symptoms of vitamin B12 deficiency can take up to 6 years to be detected in the blood (33). Long-term studies looked at the time it takes to become vitamin B12 deficient. Kumar found that metformin caused an increase in vitamin B12 deficiency after 1 year of exposure (5). Similarly, Pflipsen et al concluded that the longer a patient had been diagnosed with diabetes and taking metformin the greater risk they had of being vitamin B12 deficient (4). The same was found for dosage of metformin taken (21). Jager et al found that vitamin B12 deficiency is not a temporary issue, but is a progressive problem that will increase with time (14). In contrast, Bauman et al found a decrease in serum B12 three months after the initiation of metformin treatment (13).

Obesity and Vitamin B12 deficiency:

Only two studies related vitamin B12 deficiency to obesity (10, 16). A population-based study found that 30.5% of normal and overweight older adults were vitamin B12 deficient, while 34.4% of obese older adults were vitamin B12 deficient. A prospective descriptive study showed obese children had an average of 130 pg/mL lower serum B12 levels when compared to normal weight children. Ten percent of obese children had low serum B12 levels, while 2.2% of normal weight children had low levels (16). Neither study looked at obesity as an independent variable for vitamin B12 deficiency.

The rational for why obesity may increase the risk of vitamin B12 deficiency is unclear, but may be related to the use of heartburn medications. Heartburn, or acid reflux, is a common byproduct in adults who are overweight and obese. Many medications taken to relieve heartburn negatively affect vitamin B12 absorption. The WHO has estimated that 90% of people with Type

2 diabetes are overweight or obese (CDC, WHO) and 24% of individuals with Type 2 diabetes experience heartburn (37). NHANES has found that 18.1-27.8% of the US population regularly use Proton Pump Inhibitors for heartburn (38). High blood sugars can also relax the lower esophageal sphincter along with slowing down esophageal peristalsis (39). The relaxation of the LES sphincter can then lead to heartburn and thus increase the usage of medications.

Interventions to prevent vitamin B12 deficiency:

Vitamin B12 supplementation

Vitamin B12 supplementation includes oral cyanocobalamin, intramuscular cyanocobalamin, and oral methylcobalamin (8, 9, 15, 40). Tucker et al conducted a prospective cohort study with the offspring of the Framingham Cohort study. The participants were from a healthy population, 26 years of age or older. The study looked at oral and intramuscular vitamin B12 supplements (17). The oral supplements ranged from 1000µg/day to 1000µg/month of cyanocobalamin. The intramuscular dose varied from 1000µg/day for 1 week to 1000µg/month. The study found that individuals who took any form of vitamin B12 had an 8% risk of developing vitamin B12 deficiency while non-supplement users had a 20% risk of developing deficiency. Although oral and intramuscular treatments both found significant improvement in serum B12 levels, the study suggests using intramuscular therapy if serum B12 is <149pg/mL for the quickest results (17). NHANES found no association between a vitamin B12 deficiency risk and vitamin B12 supplementation in people over the age of 50 taking metformin. People taking up to 6µg of vitamin B12 had a 14.1% risk of becoming vitamin B12 deficient, while people taking >6µg had a 1.8% risk of developing deficiency (15).. Finally, a systematic review on vitamin B12 supplementation for individuals with diabetic peripheral neuropathy concluded that

any form of vitamin B12 supplementation would most likely not improve neuropathy measures or symptoms of the disease (40). In conclusion, the limited studies to date did not find conclusive evidence that supplementation of either calcium or vitamin B12 would decrease subjects' risk of developing vitamin B12 deficiency.

Fortified food vitamin B12 supplementation

Increasing foods high in vitamin B12, along with using fortified foods to prevent vitamin B12 deficiency has been studied. The Framingham Offspring study looked at fortified foods such as breakfast cereals and (17). The study found that individuals who consumed fortified cereal 4 times a week or more, had a 14% risk of becoming deficient while non-fortified users were 23% more likely to become deficient. The study also concluded that fortified foods such as cereal might be better absorbed than meats and other protein sources (17).

Calcium supplementation

Because calcium is needed for vitamin B12 absorption, interventions have tested calcium and vitamin B12 supplementation, through oral supplements and fortified foods, to reverse or prevent a vitamin B12 deficiency (8, 9, 13, 15, 17, 35, 40, 41). A randomized control trial of patients consuming metformin studied the use of calcium carbonate to reverse vitamin B12 deficiency (13). A calcium carbonate (1.2g/day) pill was given to participants daily after 3 months of metformin therapy. The results showed that calcium carbonate helped increase serum transcobalamin II holoTCII levels but did not significantly increase serum B12 levels. Presse et al conducted a cross sectional study on calcium supplementation of patients consuming PPIs (35). The cross sectional study collected medical charts from a Geriatric Hospital, where the calcium supplements were varied forms of calcium carbonate ranging from 250-1,500mg/day (35). The study found that only patients who took PPIs without calcium supplementation were at

risk for being vitamin B12 deficient. The study concluded that calcium supplements, along with the use of PPIs was a strong effect modifier in the prevention of vitamin B12 deficiency. Overall, the use of calcium supplementation in preventing vitamin B12 deficiency was inconclusive and more research needs to be collected.

Conclusion

Vitamin B12 deficiency is caused by medication use, age, diet and malabsorption (4, 5, 14) . The consequences of vitamin B12 deficiency include diabetic neuropathy (14), memory loss (19) and cardiovascular disease (2) . The use of the drug metformin increases the risk of vitamin B12 deficiency by 7% (14) and almost 60% of patients with vitamin B12 deficiency have a form of diabetic neuropathy (36). While causes of vitamin B12 deficiency have been researched, minimal studies have looked at the long term effects of metformin and obesity as a factor of deficiency. The use of vitamin B12 and calcium supplementation is still controversial, and there is not enough evidence to prove its effectiveness. Recent studies also suggest vitamin B12 deficiency is increasing in younger adults and even adolescents. More exploration is needed to prove cause, effect and appropriate treatment of vitamin B12 deficiency.

Chapter 3: Methods

Larger Study

Overview

The data were collected from the Activity for Diabetic Polyneuropathy: ADAPT study, a randomized clinical trial ROI: 5R01DK064814-10. The study was developed by investigators from the Physical Therapy Departments at the University of Utah Medical Center and the University of Kansas Medical Center (KUMC) with collaboration from the Department of Dietetics and Nutrition. The principal investigators are Dr. John Robinson Singleton of Utah and Dr. Patricia Kluding of KUMC. The primary aim of the ADAPT study is to determine if an integrated program of moderate supervised exercise and actigraphy, based on anti-sedentariness counseling, is an effective therapy for DPN. The second objective is to better evaluate the clinical meaning of change in intraepidermal nerve fiber density and to determine the intervention's impact on epidermal regeneration and inflammation.

Sample

Participants for the ADAPT study are recruited from patients with T2DM seen in the KU Hospital, University of Utah Hospital and community clinics and with additional recruitment efforts at affiliated clinics that conduct screenings for diabetic retinopathy. Participants are also recruited directly from the KUMC Neuromuscular clinics and Veterans Administration Centers as appropriate. Provider letters and clinic fliers are utilized to enhance enrollment. Inclusion criteria includes men or women between 30-70 years of age with T2DM defined by American Diabetes Association (ADA) criteria (42). They must have peripheral neuropathy, based on the Toronto Diabetic Neuropathy Expert group criteria (43), and be under the care of an identified Primary Care Physician. Exclusion criteria include any alternative cause for peripheral

neuropathy. Individuals with obesity, hypertension, diabetes or hyperlipidemia considered in a dangerous range (Body Mass Index (BMI) >45, HgbA1c >10, systolic BP >170, or diastolic BP >110, and those with triglycerides > 750 mg/dL) are also excluded.

Other enrollment criteria include vitamin B12, thyroid stimulating hormone, antinuclear antibody, serum protein electrophoresis and immunoflixation laboratory values all normal within the last 12 months. A final exclusion criteria for the ADAPT study is having low serum B12 levels; therefore, participants in this study are not vitamin B12 deficient. Individuals with serum B12 levels <200ng/L at screening are required to supplement with 1-2 mg of oral vitamin B12 for three months before recheck. Individuals with serum B12 levels of 200-299 ng/L are required to test their methymalonic acid blood levels. If they are elevated, then oral vitamin B12 supplementation is required for three months prior to rescreening. Individuals with serum B12 levels >300 ng/L are accepted into the study.

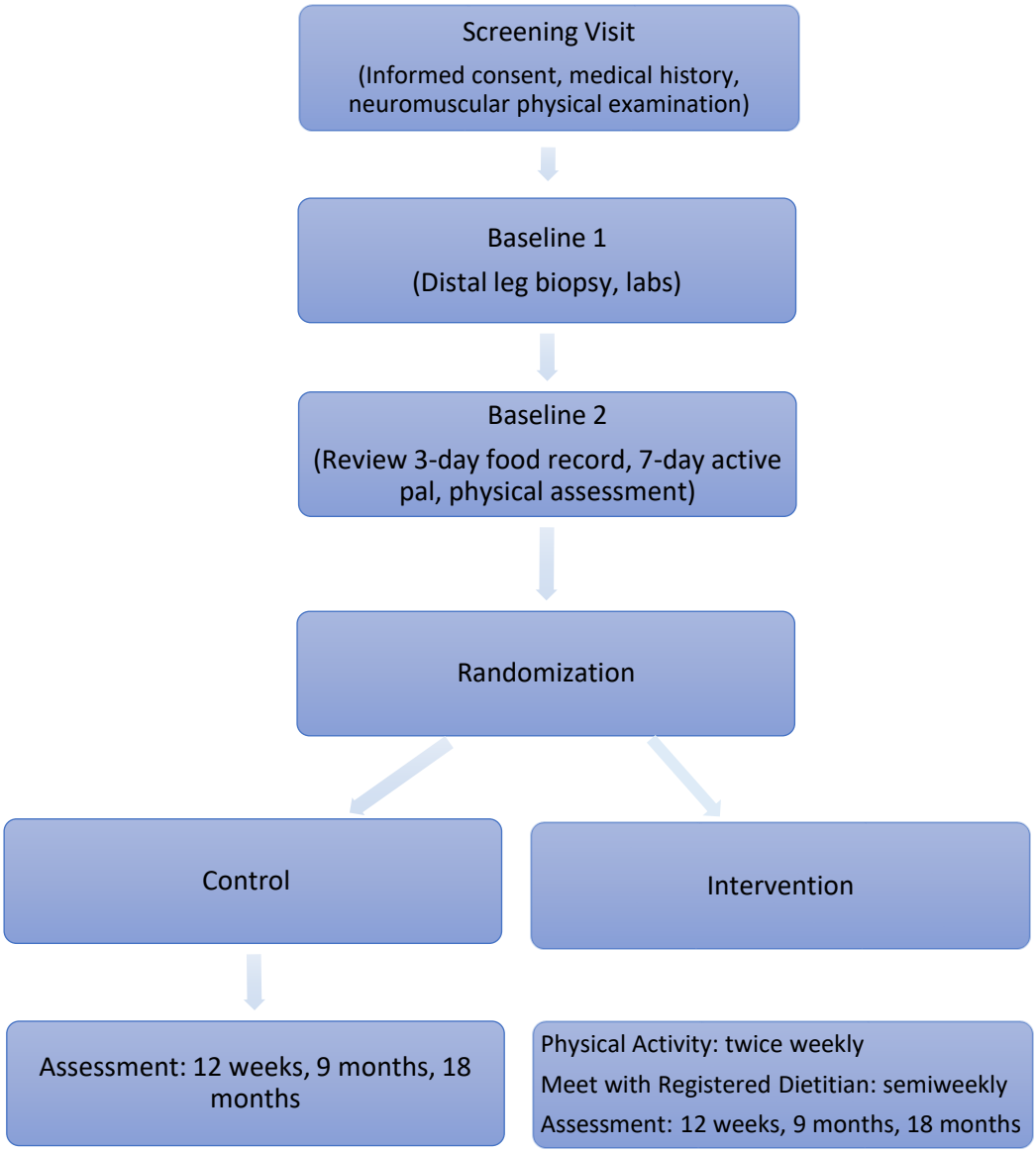
Setting

The study is conducted through the University of Kansas Medical Center and the University of Utah Medical Center. Recruitment will occur for 42 months and each participant will be enrolled for 18 months.

Ethics

The Human Subjects Committee at both KUMC and Utah approved this proposal. Written informed consent is obtained. Participants are compensated $50 for completing each of the following visits: Baseline 1, Baseline 2, Month 3, Month 9 and Month 18, for up to a maximum of $250.

Procedure


Screening Visit
(Informed consent, medical history, neuromuscular physical examination)
Baseline 1
(Distal leg biopsy, labs)
Baseline 2
(Review 3-day food record, 7-day active pal, physical assessment)
Randomization
Control
Intervention
Assessment: 12 weeks, 9 months, 18 months
Physical Activity: twice weekly
Meet with Registered Dietitian: semiweekly
Assessment: 12 weeks, 9 months, 18 months

Larger ADAPT Study Methods

Screening and Baseline 1

During the screening process, after written informed consent is obtained, the investigator takes a medical, family and neurological history via forms created by the University of Utah. A vitals assessment of pulse, heart rate and blood pressure, and a focused, standardized neuromuscular physical examination, which incorporates the Utah Early Neuropathy Scale (UENS), a standardized neuropathy exam scale (44) is administered. Once individuals are approved, they participate in the Baseline 1 visit. During Baseline 1, the following procedures are performed to assure they meet the entry criteria and have the qualifications of "Peripheral Neuropathy," measured by a distal leg skin biopsy, physician supervised graded maximal exercise test and laboratory tests which include HbgA1c, fasting lipid panel, serum vitamin B12, thyroid stimulating hormone and antinuclear antibody labs.

Demographics and Medication use

Age and medication usage is collected from the participants by self-report on Utah survey forms along with their medical chart provided by their Primary Care Provider.

Serum Vitamin B12

The blood was drawn by a nurse at the Clinical and Translational Science Unit, processed and shipped to The University of Utah for analyses. Serum B12 was measured in mg/dL. The results are added to the participants' personal binder and RedCap database.

Anthropometrics

Participants had their height, weight and waist circumference measured by the blinded Baseline 1 assessment team. Weight was measured in kilograms using a digital weight scale. Participants wear their normal clothes and the removal of shoes is voluntary. The team notes, on the assessment form, whether or not shoes are removed. The weight appears on the screen and the measurement is recorded on the assessment form. For assessment of height, participants are asked to stand up straight against the stadiometer backboard with their body weight evenly distributed and both feet flat on the platform. They are instructed to stand with their heels together and toes apart. The team checks to ensure the back of the head, shoulder blades, buttocks, and heels make contact with the backboard. The stadiometer headpiece is lowered so it rests firmly on top of the participants' heads with sufficient pressure to compress their hair. Participants are instructed to stand as tall as possible, take a deep breath and hold the position. A single measurement is taken, and the team records the height along with whether the subject is wearing shoes.

Waist circumference is measured by having participants stand with feet shoulder width apart and arms crossed over their chests. The hips are palpated to locate the top of the iliac crest and a line is drawn at this spot between their back and abdomen. The measuring tape is placed horizontally around the participants' abdomens. A spring hand measuring tape is used to control pressure exerted on their abdomen. The participants are instructed to relax and take 3 normal breaths. The measure is taken from the zero line of the tape at the end of a normal expiration. Waist circumference is only measured once.

BMI is calculated from height and weight: bodyweight in kilograms divided by height in meters squared. Individuals with a BMI of 18.5 -24.9kg/m^2 are classified as normal weight,

25.0-29.9 kg/m^2as overweight, and 30.0- 45kg/m^2 as obese. All anthropometric data are added to the participants' personal binder and RedCap database.

Baseline 2

Once participants pass Baseline 1 requirements, they complete the Baseline 2 visit. In preparation for the Baseline 2 evaluation, participants are asked to complete a standard 3-day food record over a period spanning two weekdays and one weekend. During the visit, participants discuss their completed three-day food record with a registered dietitian, review weight and metabolic lab results, and receive uniform basic nutritional counseling from the registered dietitian. The standardized 20-minute session provides information based on the Diabetes Prevention Program goals: reduce total calories and fat calories to achieve weight loss.

Dietary Intake

A 3-day food record document was created by the University of Utah and can be found in the appendix. The record consists of directions and examples on how to complete a 3-day food record. The University of Utah's registered dietitians created the record and trained the KUMC registered dietitian via email on how to collect the record. The KUMC registered dietitian provided training to the Baseline 1 assessment team on how to explain the diet record to the participants at the Baseline 1 visit. Participants are required to fill out all food and beverages consumed over a consecutive 3 day period of two weekdays and one weekend. They are instructed to include time of day, location, and as many details of the meal consumed, including preparation and any additional ingredients added to the meal.

Participants meet with the registered dietitian for the first time during the Baseline 2 visit. The registered dietitian guides the participants through all three days of the food record to make

sure all food and beverages consumed are included and obtains clarifications as needed. The registered dietitian then asks for any additional dietary, herbal or other supplements taken and adds it to the supplement section on the record. Once the record is complete, the registered dietitian enters the food records into the Food Processor 10.15.0 database structure version 9.8.2.

Randomization

Once both baseline visits are completed, participants are randomized into two groups: the intervention group, which receives personalized dietary counseling every other week for the first 12 weeks and monthly thereafter, and the control group which receives no additional counseling. The intervention group meets twice weekly for the first 12 weeks. Goals include aerobic exercise of 70% VO2 reserve for 50 minutes; 3 sets of 15 repetitions of 3 upper and 3 lower body strength exercises at 60-70% of repetition maximum twice a week. Participants are then given standard counseling by a registered dietitian on diet (target of 7% weight reduction) every other week for the first 12 weeks. Weight and waist circumference are obtained at each visit and charted as a motivational aid. The dietary goals are evaluated and feedback is provided at least once every other week.

Present Study Methods

Subjects

Participants in the present study included 16 men and 14 women. Fifteen of the participants were from Utah and fifteen were from KUMC.

Materials

Three-day diet records, body weight, anthropometrics measures, serum vitamin B12 values and statistical analysis software were needed to complete the study. For this purpose of the present study, the 3-day diet records were entered into a nutrient analysis software program, Nutrition Data System for Research (NDSR) software version 2016, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. The records were entered into NDSR for the purpose of the present study because the database does not allow missing values for nutrients while other databases allow missing values.

Analysis of Data

The anthropometric data along with medications were pulled from a RedCap database where it had been entered by the KUMC and Utah assessment teams. Results from the 3-day food records were pulled from NDSR. Baseline demographics and outcome variables were summarized using descriptive statistics. A one-way analysis of variance (ANOVA was used to compare individuals taking metformin and non-users). A regression analysis was used to determine the relationship between serum B12 and the following variables: dietary vitamin B12 (food and beverages), BMI, age and vitamin B12 supplement usage.

The baseline 3-day food records were entered in NDSR by a registered dietitian trained in NDSR data entry. All 3-day food records entered, were verified by an additional NDSR trained

registered dietitian for quality assurance prior to analyzing data. The NDSR Foods 2016 Database Documentation file and output files 01, 04 and 12 were used for the analyses.

To determine the average daily intake (µg) of vitamin B12 in adults with diabetic peripheral neuropathy, the average of all dietary sources of vitamin B12 was calculated using the Intake Properties Totals file (04) and the average of all supplement sources of vitamin B12 was calculated using the DSAM Total 24-hour Supplement Intake file (12) for each participant. The average daily intake vitamin B12 from dietary and supplement sources were then combined for a total oral vitamin B12 intake. To assess dietary sources of vitamin B12, the Component/Ingredient file (01) was sorted to categorize each food and beverage into 3 source types: animal, fortified food and beverage, or miscellaneous sources. The Foods 2016 Database Documentation file was used to assist in identifying specific foods and beverages for each category by utilizing the NDSR food descriptions and Food IDs columns. The average daily intake of vitamin B12 was calculated for each category by participant and by group. Daily total macronutrient distribution and total calories consumed for each participate were totaled and averaged. A t-statistic was used to evaluate variances between metformin and non-metformin participants.

Chapter 4: Results

The purpose of the present study was to determine the average daily intake of vitamin B12 in adults with diabetic peripheral neuropathy and whether there was a difference in serum vitamin B12 concentrations between those taking the medication metformin vs. non-users. In addition, the study aimed to answer whether there was a relationship between vitamin B12 consumption and serum B12 levels; Body Mass Index and serum B12; and age and serum B12 levels.

Thirty participants, fifteen from Utah and fifteen from KUMC each had a 3-day food record along with baseline demographics, medication and supplement usage. The mean age of the sample was 59.8 ± 7.03 years with ages ranging from 38 to 70 years old. The study consisted of 16 men and 14 women. The mean Body Mass Index was 33.45 ± 6.32 kg/m^2 (range 21.5 to 51.4). Twenty-three (77%) of the subjects had a BMI greater than 30 kg/m^2, categorizing them as obese. The average serum B12 level was 650.67 pmol/L ± 413.58 pmol/L with levels ranging from 222 pmol/L to >2000 pmol/L. Table 1 depicts the characteristics of the participants.

Seventy percent (21/30) of participants were on the drug metformin and 37% were taking a vitamin B12 supplement. There was no difference between metformin users and non-users in age, BMI, supplement use and serum B12 levels. Refer to Table 1.

Table 1: Demographics and Differences between Metformin Users vs. Non-Users

		Medication Use		
	Total Sample (n=30)	Non-Metformin Users (n=9)	Metformin Users (n=21)	p-value
Age (years)	59.8 ± 7.0	59.6 ± 4.2	59.9 ± 8.0	0.88
BMI (kg/m^2)	33.5 ± 6.3	34.28 ± 6.2	33.1 ± 6.5	0.64
Serum Vitamin B12 (pg/mL)	650.7 ± 413.6	806.1 ± 492.7	584.0 ± 367.9	0.24
Vitamin B12 Supplement dose (µg/d)	92.1 ± 455.1	8.6 ± 16.3	127.9 ± 543.8	0.32
Metformin Dose (mg/d)	1095.0 ± 885.7	_	1564.3 ± 543.8	0.182

The average energy intake from the 3 day food records was 1,728 kcal per day. Macronutrient distribution ranges were 42% of the energy from carbohydrate, 37% from fat and 17% from protein. The mean dietary vitamin B12 intake was 4.3 µg. Forty three percent (43%) of total dietary vitamin B12 came from animal sources, while 20% was derived from fortified foods. One subject was a vegetarian and only 48% of the participants consumed foods fortified with vitamin B12. Metformin users had a significantly lower percent of energy from protein versus non-users (16.1% vs. 19.6%, respectively). No other significant differences were observed between the groups. Refer to Table 2 for the dietary intake data.

Table 2: Dietary Intake

		Medication Use		
Variables	Total (n=30)	Non-Metformin (n=9)	Metformin (n=21)	p-value
Energy (kcal)	1728.3 ± 504.8	1547.2 ± 324.5	1805.8 ± 553.4	0.12
Fat (g)	73.6 ± 22.9	69.2 ± 13.9	75.5 ± 25.9	0.39
Carbohydrate (g)	188.1 ± 61.9	163.9 ± 57.4	198.5 ± 71.9	0.16
Protein (g)	72.3 ± 22.9	73.3 ± 24.5	71.8 ± 4.17	0.88
Percent Fat (%)	37.3	38.8	37.2	0.53
Percent Carbohydrate (%)	43.1	41.2	43.8	0.43
Percent Protein (%)	17.1	19.6	16.1	0.04
Total Vitamin B12 (µg)	4.3 ± 2.6	4.6 ± 2.2	4.2 ± 2.8	0.67
Vitamin B12 from Animal Sources (µg)	3.4 ± 1.9	3.9 ± 1.5	3.1 ± 2.0	0.27
Vitamin B12 from Fortified Foods (µg)	0.91 ± 1.5	1.4 ± 2.3	0.69 ± 1.0	0.39
Vitamin B12 for Misc. (µg)	0.07 ± 0.10	0.05 ± 0.07	0.08 ± 0.1	0.42
Vitamin B12 from Supplements (µg)	92.0 ± 455.1	4.8 ± 6.7	127.8 ± 543.8	0.32
Total Vitamin B12 from Diet and Supplements (µg)	94.9 ± 455.1	10.2 ± 10.6	131.2 ± 547.0	0.32

There was no significant difference in serum B12 levels between metformin users and non-users (p= 0.182). There was also no relationship between total dietary intake of vitamin B12

and serum vitamin B12 concentration (p= 0.17). However, there was a significant relationship between vitamin B12 supplement use and serum vitamin B12 (p=0.031). There were also no significant relationships between serum vitamin B12 and the demographics of age, BMI, or obesity status. Table 3 shows the results from the regression analysis.

Table 3: Regression Analysis for Independent Variables

	Serum B12 pg/mL			
Variable	*S*	*SE B*	B	*p-value*
Dietary B12 (µg)	-41.2	29.3	-0.25	.170
BMI (kg/m²)	-1.3	12.4	-0.2	.9150
Age (years)	10.7	10.9	.181	.338
Supplements (µg)	.359	.158	.395	.031*

A pairwise correlation matrix was used to look at the relationship of each independent variable. Table 4 shows the statistical significance of supplement use with serum B12. The table also illustrates the correlation of fortified foods and animal sources to overall vitamin B12 consumption. Fortified foods (p= 0.00) had a stronger correlation to overall vitamin B12 intake than animal sources (p=0.016)

Table 4: Pairwise Correlation. Sig (2-tailed), p-value

	1	2	3	4	5	6	7	8
1. Age (years)								
2. BMI (kg/m²)	-.275 .141							
3. Serum B12 (pg/mL)	-.181 .338	-.020 .915						
4. Metformin (mg)	.006 .974	.078 .684	-.097 .611					
5. Supplements (µg)	.168 .374	-.105 .580	-.395 .031*	.197 .296				
6. Fortified Foods (µg)	.031 .871	-.251 .180	-.193 .307	-.040 .835	-.022 .907			
7. Animal Sources Sources (µg)	-.076 .689	-.059 .758	-.234 .213	.200 .288	-.124 .512	.226 .229		
8. Dietary B12 (µg) + Supplements (µg)	.168 .375	-.106 .575	.394 .031*	.198 .294	1.00 .000*	-.018 .925	-.120 .528	
9. Total Dietary B12 (µg)	-.049 .796	-.203 .283	-.257 .170	.146 .441	-.063 .740	.745 .000*	.810 .000*	-.058 .762

*correlation is significant at the 0.05 level (2-tail)

Chapter 5: Discussion

Overall Daily vitamin B12 intake

The average daily dietary intake (µg) of vitamin B12 in the present study was 4.3 µg per day. The Estimated Average Requirements (EAR) for men and women age 30-70 years of age is 2.0 µg per day. Data collected from the 3-day food records revealed five individuals did not meet the EAR for vitamin B12 which indicates a high probability that their diets are inadequate in vitamin B12. These results differ significantly from the Framminghам offspring study that found average dietary B12 consumption in adults 26-83 years of age was 8.7 ± 0.3 µg per day (17). The Framminghам offspring population reported by Tucker et al did not have diabetes and dietary intake was collected using a semi quantitative 126 question food frequency questionnaire and calculated total vitamin B12 intake including supplements, fortified foods and animal sources. The present study collected dietary intake via a 3-day food record and supplement intake. The type of food record utilized, sample population characteristics and inclusion of supplements could explain differences in results. Tucker et al did not publish average energy consumption for comparison which could also be a confounding variable. Few vitamin B12 studies have collected food records from participants, therefore our study could show a more accurate description of average vitamin B12 intake. Regardless, the results call for further research to explore the validity and mechanism behind the varying results.

Metformin

De Jager et al and Kumar et al found that taking metformin significantly lowered serum vitamin B12 in patients with diabetic neuropathy more than those not taking metformin by 19% (5, 14). Kumar et al conducted a cross-sectional study similar to the present study looking at

participants with T2DM consuming and not consuming metformin(5) He found that mean serum B12 was lower in the group taking metformin (410mg/dL ± 230.7 vs 549.2 ± 244.7mg/dL). De Jager et al conducted a randomized placebo control trial that found participants with T2DM who started on metformin developed a fall in serum B12 levels by an average of 89.8pmol/L (14). The study also looked at risk of vitamin B12 deficiency with the use of metformin. Analysis of participant data in the present study did not control for dosage of metformin or length of use. Despite the similarities in the studies, the present study did not find the same association as de Jager and Kumar. The difference between the previous studies and the present study cannot be determined from these data. However, it can be speculated that it may be due to the duration of metformin use and sample size. For example, NHANES 1999-2002 separated metformin use by years (15). Participants that had taken metformin >10 years had the highest prevalence of serum B12 deficiency. A further explanation may be the fact that individuals with low serum vitamin B12 were excluded from participation in the present study.

Vitamin B12 consumption

The present study supports the findings from other studies that taking a vitamin B12 supplement has a positive relationship with serum B12 (10, 15, 17). El-Khateeb found taking a B-Complex supplement protected against vitamin B12 deficiency in individuals with T2DM (10). Tucker et al from the Framingham Offspring study found that any supplement containing vitamin B12 protected against deficiency, while NHANES data indicated that vitamin B12 supplementation decreased prevalence of deficiency(15). The present study strengthens the recommendation for individuals with diabetes to consume a vitamin B12 supplement to protect against deficiency.

Body Mass Index

The present study did not find a positive relationship between BMI or obesity status and serum vitamin B12 levels. This contradicts data from the NHANES 1999-2006 survey in which a positive relationship was reported between serum B12 levels and a BMI of 25-30kg/m^2 (15). Similarly El-Khateeb et al in their population based study found obese individuals ($\geq$ 40 kg/m^2) to have the highest rates of serum vitamin B12 deficiency (10). The NHANES sample size was 8,488 participants and El-Khateeb collected data on 5,640 individuals. The differences could be explained by the small sample size of only 30 participants as well as the exclusion criteria of individuals with a serum vitamin B12 <300pmol/L. Further, NHANES data revealed significant relationships in overweight individuals while El-Khateeb found significant relationships only in obese individuals. The two studies do not share a consensus on weight in relation to vitamin B12 and both studies could have confounding variables, such as PPI use. Finally, the present study has 73% of the subjects distributed into the obese category. El-Kahteeb included individuals with a BMI >40 kg/m^2 while this group was excluded in the present study. A more evenly distributed sample size might have affected the results.

Age

Solomon et al, Couderc et al and Reinstatler et al found that the prevalence of vitamin B12 deficiency increases with age (8, 15, 41). This is partially due to a decrease in intrinsic factor production and food-cobalamin malabsorption (8). Our study did not find a relationship between age and serum vitamin B12 levels.

El-Khateeb et al found in his population based study that individuals between the ages of 19-39 year of age had the highest rate of vitamin B12 deficiency (10). Our study did not have

any individuals under the age of 38, but the data could strengthen El-Khateeb's study in showing that age is not a factor for vitamin B12 deficiency. The present study, as well as data from El-Khateeb's study, could represent new findings in age and its relationship to serum B12 levels. More research studying the relationship between age and serum B12 levels is needed. Nevertheless, based on recent data, testing for deficiency starting at a younger age would be recommended.

Supplementation

The present study found that supplements containing vitamin B12 have a positive relationship with serum vitamin B12 levels. This compares to the NHANES 1999-2000 survey that found, independent of vitamin B12 dosage, taking a supplement will decrease your risk of vitamin B12 deficiency (15). Similarly Pflipsen et al states that a multivitamin protects individuals with diabetes from vitamin B12 deficiency (4). Only 37% of the individuals in the present study were taking a supplement containing vitamin B12. The Institute of Medicine suggests individuals over the age of 50 should consume either vitamin B12 fortified foods or a supplement containing vitamin B12 to meet their RDA(29). The present study supports previous research that supplementation protects against low serum B12 levels.

Limitations

There are several limitations to the present study. It is a secondary analysis using previously collected data. Therefore, the information available is limited and there is no opportunity for further clarification of dietary records, weights, etc. In addition, the dietary intake data are self-reported which are known to have flaws. Supplement use is collected from

participants at Baseline 1. Their use of supplements could be inconsistent and could provide false information on vitamin B12 supplement usage.

Human error can occur during data entry into NDSR, the software program used for 3-day food records. However, we tried to limit coding errors by having all the data entry verified by a second qualified diet entry coder. The study is split between two universities, with separate teams collecting data including anthropometric measurements. Discrepancies of data collection could have occurred among the two universities.

The study demographics are not evenly distributed. Twenty-three out of thirty (77%) of the individuals were in the obese category, while twenty-one out of thirty (70%) of the individuals took metformin. The amount of overweight and obese subjects could have affected the analysis of BMI to serum vitamin B12 along with metformin use to serum vitamin B12.

The sample size was small and statistical significance may be weak compared to a study with a larger sample size. Finally, a major exclusion criterion was having a serum B12 level under 299mg/dL. Therefore, individuals in the present study were not vitamin B12 deficient.

Chapter 6: Summary

Previous studies have had conflicting results regarding age, BMI, medication use and supplements on their impact on serum B12 levels (5, 14, 17). Furthermore, few studies have looked specifically at individuals with diabetic peripheral neuropathy and their serum B12 levels. No studies reviewed have used 3-day food records to analyze total nutrient intake, macronutrient and micronutrient distribution. Many studies focus on medication and demographics vs. supplementation (4, 14, 15). The most recent study, El-Khateeb et al (2014), concluded that there was not a relationship between old age and serum B12 (10). They also stated that there is a positive relationship between BMI and serum B12 (10). Although the present study did not find a relationship between BMI and serum B12, it did concur with El-Khateeb's finding that old age did not increase the risk of vitamin B12 deficiency. Thus, the present study strengthens El-Khateeb's more recent findings.

The present study will hopefully fill the gaps of previous research by specifically looking at dietary and supplement B12 consumption and serum B12 levels. More research is needed for the specific analysis and relationship of animal sources vs fortified food sources on serum B12 levels. The present study can strengthen new research on serum B12 deficiency and the relationship of serum B12 on supplementation.

Literature Cited

1. Prevention CDCa. National Diabetes Statistics Report: Estimates of Diabetes and It's Burden in the United States. In: Services DoHaH, ed. Atlanta, GA, 2014.
2. Organization WH. Internet: http://www.who.int/dietphysicalactivity/media/en/gsfs_obesity.pdf.
3. Health NIo. Diabetic Neuropathies: The Nerve Damage of Diabetes. In: Services USDoHaH, ed. NIH Publication: NIH, 2009.
4. Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B(12) deficiency in patients with type 2 diabetes: a cross-sectional study. J Am Board Fam Med. 2009;22(5):528-34. doi: 10.3122/jabfm.2009.05.090044.
5. Singh AK, Kumar A, Karmakar D, Jha RK. Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients. J Postgrad Med. 2013;59(4):253-7. doi: 10.4103/0022-3859.123143.
6. Andrès E, Loukili NH, Noel E, Kaltenbach G, Abdelgheni MB, Perrin AE, Noblet-Dick M, Maloisel F, Schlienger J-L, Blicklé J-F. Vitamin B12 (cobalamin) deficiency in elderly patients. Can Med Assoc J. 2004;171(3):251-9. doi: 10.1503/cmaj.1031155.
7. Lindenbaum J, Rosenberg IH, Wilson PW, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. Am J Clin Nutr. 1994;60(1):2-11.
8. Couderc AL, Camalet J, Schneider S, Turpin JM, Bereder I, Boulahssass R, Gonfrier S, Bayer P, Guerin O, Brocker P. Cobalamin deficiency in the elderly: aetiology and management: a study of 125 patients in a geriatric hospital. J Nutr Health Aging. 2015;19(2):234-9. doi: 10.1007/s12603-014-0525-1.
9. Solomon LR. Diabetes as a cause of clinically significant functional cobalamin deficiency. Diabetes Care. 2011;34(5):1077-80. doi: 10.2337/dc11-0009.
10. El-Khateeb M, Khader Y, Batieha A, Jaddou H, Hyassat D, Belbisi A, Ajlouni K. Vitamin B12 Deficiency in Jordan: A Population-Based Study. Ann Nutr Metab. 2014;64(2):101-5 5p. doi: 10.1159/000355440.
11. Yajnik CS, Deshpande SS, Lubree HG, Naik SS, Bhat DS, Uradey BS, Deshpande JA, Rege SS, Refsum H, Yudkin JS. Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. J Assoc Physicians India. 2006;54:775-82.
12. Bell DS. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. South Med J. 2010;103(3):265-7. doi: 10.1097/SMJ.0b013e3181ce0e4d.
13. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care. 2000;23(9):1227-31.
14. de Jager J, Kooy A, Lehert P, Wulffele MG, van der Kolk J, Bets D, Verburg J, Donker AJ, Stehouwer CD. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ. 2010;340:c2181. doi: 10.1136/bmj.c2181.
15. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP, Jr. Association of biochemical B12 deficiency with metformin therapy and vitamin B12 supplements: the

national health and nutrition examination survey, 1999-2006. Diabetes Care. 2012;35(2):327-33 7p.
16. Pinhas-Hamiel O, Doron-Panush N, Reichman B, Nitzan-Kaluski D, Shalitin S, Geva-Lerner L. Obese children and adolescents: a risk group for low vitamin B12 concentration. Arch Pediatr Adolesc Med. 2006;160(9):933-6. doi: 10.1001/archpedi.160.9.933.
17. Tucker KL, Rich S, Rosenberg I, Jacques P, Dallal G, Wilson PW, Selhub J. Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. Am J Clin Nutr. 2000;71(2):514-22.
18. Roglic G. Internet: http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf?ua=1 (accessed 7/20/2016 2016).
19. NIDDK. Internet: https://www.niddk.nih.gov/health-information/health-topics/Diabetes/diabetic-neuropathies-nerve-damage-diabetes/Pages/diabetic-neuropathies-nerve-damage.aspx (accessed 7/20/2016 2016).
20. Dali-Youcef N, Andres E. An update on cobalamin deficiency in adults. QJM. 2009;102(1):17-28. doi: 10.1093/qjmed/hcn138.
21. Golay A, Ybarra J. Link between obesity and type 2 diabetes. Best Practice & Research Clinical Endocrinology & Metabolism. 2005;19(4):649-63. doi: http://dx.doi.org/10.1016/j.beem.2005.07.010.
22. Prevention CfDCa. Internet: http://www.cdc.gov/diabetes/basics/diabetes.html 2016).
23. Thomas RACCBCJRTLKZR. Modern Nutrition in Health and Disease. 11 ed. Philadelphia, PA: Lippincotte Williams and Wilkins, 2014.
24. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. J Clin Invest. 2007;117(5):1219-22. doi: 10.1172/JCI32169.
25. Boulton AJM, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D. Diabetic Neuropathies. A statement by the American Diabetes Association. 2005;28(4):956-62. doi: 10.2337/diacare.28.4.956.
26. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clinical Science (London, England : 1979). 2012;122(6):253-70. doi: 10.1042/CS20110386.
27. Jones A. Vitamin B12 levels in type 2 diabetes patients taking metformin. Practice Nurse. 2012;42(10):37-9 3p.
28. Morell P QR. Basic Neurochemistry: Molecular, Cellular and Medical Aspects 6th Edition. The Myelin Sheath Philadelphia: Lippincott-Raven, 1999.
29. Standard G. March 27. Internet: https://www-clinicalkey-com.proxy.kumc.edu/#!/content/drug_monograph/6-s2.0-154?scrollTo=%23References (accessed 06/22/2016 2016).
30. Supplements OoD. Internet: https://ods.od.nih.gov/factsheets/VitaminB12-HealthProfessional/ (accessed 06/22/2016 2016).
31. Harrington DJ. Laboratory assessment of vitamin B12 status. J Clin Pathol. 2016. doi: 10.1136/jclinpath-2015-203502.
32. Carmel R. Cobalamin, the stomach, and aging. Am J Clin Nutr. 1997;66(4):750-9.
33. Chatthanawaree W. Biomarkers of cobalamin (vitamin B12) deficiency and its application. Journal of Nutrition, Health & Aging. 2011;15(3):227-31 5p. doi: 10.1007/s12603-010-0280-x.

34. Clarke R, Sherliker P, Hin H, Nexo E, Hvas AM, Schneede J, Birks J, Ueland PM, Emmens K, Scott JM, et al. Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. Clin Chem. 2007;53(5):963-70. doi: 10.1373/clinchem.2006.080382.
35. Presse N, Perreault S, Kergoat M-J. Vitamin B12 deficiency induced by the use of gastric acid inhibitors: Calcium supplements as a potential effect modifier. Journal of Nutrition, Health & Aging. 2016;20(5):569-73 5p. doi: 10.1007/s12603-015-0605-x.
36. Dyck PJ, Kratz KM, Karnes JL, Litchy WJ, Klein R, Pach JM, Wilson DM, O'Brien PC, Melton LJ, 3rd, Service FJ. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. Neurology. 1993;43(4):817-24.
37. Maleki D, Locke G, Iii, Camilleri M, et al. GAstrointestinal tract symptoms among persons with diabetes mellitus in the community. Arch Intern Med. 2000;160(18):2808-16. doi: 10.1001/archinte.160.18.2808.
38. Czwornog JL, Austin GL. Association of Proton Pump Inhibitor (PPI) Use with Energy Intake, Physical Activity, and Weight Gain. Nutrients. 2015;7(10):8592-601. doi: 10.3390/nu7105416.
39. Zhang Q, Horowitz M, Rigda R, Rayner C, Worynski A, Holloway RH. Effect of hyperglycemia on triggering of transient lower esophageal sphincter relaxations. Am J Physiol Gastrointest Liver Physiol. 2004;286(5):G797-803. doi: 10.1152/ajpgi.00383.2003.
40. Jayabalan B, Low LL. Vitamin B supplementation for diabetic peripheral neuropathy. Singapore Med J. 2016;57(2):55-9. doi: 10.11622/smedj.2016027.
41. Solomon LR. Advanced Age as a Risk Factor for Folate-Associated Functional Cobalamin Deficiency. J Am Geriatr Soc. 2013;61(4):577-82 6p. doi: 10.1111/jgs.12155.
42. American Diabetes A. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2010;33(Suppl 1):S62-S9. doi: 10.2337/dc10-S062.
43. Tesfaye S, Boulton AJM, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, et al. Diabetic Neuropathies: Update on Definitions, Diagnostic Criteria, Estimation of Severity, and Treatments. Diabetes Care. 2010;33(10):2285-93. doi: 10.2337/dc10-1303.
44. Singleton JR, Bixby B, Russell JW, Feldman EL, Peltier A, Goldstein J, Howard J, Smith AG. The Utah Early Neuropathy Scale: a sensitive clinical scale for early sensory predominant neuropathy. J Peripher Nerv Syst. 2008;13(3):218-27. doi: 10.1111/j.1529-8027.2008.00180.x.

Appendices

Appendix A: Food Diary 3-day food log

ADAPT
Activity for Diabetic Polyneuropathy

Subject Initials: _ _ _ Subject #: 104 - _ _ _ _

3-DAY FOOD LOG (To be completed over 2 weekdays and 1 weekend day, during 7 days prior to baseline visit)

DIRECTIONS FOR FOOD DIARY:

Record everything that you eat or drink over a three day period, two weekdays and one weekend day. This record should include all food and supplements and all water or other liquids consumed.

1. Record all food and drink whether eaten/given at home or away from home.
2. Be as specific as possible in recording the item, and use a brand name if available and include a label for nutrient information.
3. Measure the item when possible or estimate the amount using the pictures provided with this document.

   You can record in cubic centimeters (cc) or ounces (oz.) with regard to liquids and either ounces (oz.) or grams (g) for food items.
4. Please mention, if possible how the food was prepared (fried, boiled, baked, microwaved, etc.) and record items used in the preparation, including oil, margarine or butter, for instance.
5. Don't forget to include all condiments, such as catsup, gravy, sauce, butter or oil, and record amounts as accurately as possible. For instance, you might record 2 French fries with 1 teaspoon catsup.
6. Please include one item per line where possible.
7. You can use abbreviations for measurements:
8. **RETURN THIS FORM AT YOUR NEXT VISIT** :_____/_____/_____

Teaspoon – tsp ¼ cup – ¼ C Tablespoon – Tbsp. Ounce – oz.

EXAMPLE: ADULT'S DIET (Record only what is eaten)

Time	FOOD and FLUID Consumed (Description/Preparation)	Amount
5:00 pm	Chicken breast/grilled	4 oz.
5:00 pm	Canned no salt added green beans, microwaved	1 cup
5:00 pm	Medium white dinner roll(3 in diameter),homemade	1 each
5:00 pm	I can't believe it's not butter spread	1 tsp.