CHANGES IN VITAMIN B12, HOMOCYSTEINE, AND NEUROLOGICAL FUNCTION IN OLDER ADULTS

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CHANGES IN VITAMIN B12, HOMOCYSTEINE, AND NEUROLOGICAL FUNCTION IN OLDER ADULTS

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Vitamin B12 deficiency affects 5-20% of older adults, with up to 40% with low B12 levels [<260pmol/L]. The risk of B12 deficiency increases with age. Vitamin B12 is essential for neurological function, and B12 deficiency is a known cause of clinical neuropathy and has been associated with cognitive impairment. However, little is known about the relationship between low B12 levels and peripheral nerve function and information processing speed, cross-sectionally or longitudinally, in older adults. The purpose of this dissertation is to examine whether low B12 levels or change in B12 levels were associated with peripheral nerve function or information processing speed. We examined whether low B12 or high homocysteine ≥[3µmol/L] and 3-year change in B12 and homocysteine was associated with 6-year change in peripheral nerve function or neurological signs in older Italian adults from the InCHIANTI Study. We found high homocysteine was associated with lower nerve conduction amplitude and greater inability to detect 4g monofilament. In the Health ABC Study, we studied whether low [<260pmol/L] or deficient [<260pmol/L & methylmalonic acid [MMA] >271nmol/L & MMA>2-methylcitrate] B12 were associated with peripheral sensory and motor nerve function, and evaluated whether there was a threshold effect of serum B12 levels on peripheral nerve function in older black and white adults. We found poor B12 were associated with greater insensitivity to 1.4g monofilament and worse nerve conduction velocity [NCV] and there was a significant serum B12 threshold level of 390 pmol/L for NCV. To consider cognitive function, we examined

whether low B12 or 7-year change in B12 were associated with decline in Digit Symbol Substitution Test [DSST] scores over 6-years. We found low B12 was associated with greater DSST decline and a serum B12 level of 410 pmol/L was associated with lower DSST decline. These results have important public health significance, because low B12 levels, above clinical deficiency [<148 pmol/L], were associated with worse peripheral nerve function and decline in information processing speed, which may lead to decreased cognitive and physical function, and disability in older adults. Vitamin B12 supplementation is widely available, adequately absorbed, well-tolerated, and potentially may prevent declines in neurological function.

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PREFACE

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1.0 INTRODUCTION

Vitamin B12, also known as cobalamin, is a coenzyme (Stover 2010). It contains 5 nitrogen-cobalt bonds and 1 cobalt-carbon bond. Dietary forms of cobalamin include methylcobalamin and 5-deoxyadenosylcobalamin. Synthetic forms include cyanocobalamin and hydroxycobalamin.

1.1 EPIDEMIOLOGY

About 5-20% of older adults are deficient in vitamin B12 (Stabler 1999; Park 2006; Varela-Moreiras 2009), with up to 40% with low B12 levels (Baik 1999). Studies have reported a wide range of prevalence rates, due to different populations studied, but also because of a lack of agreement on diagnostic criteria for low or deficient B12. The prevalence of vitamin B12 deficiency increases with age (Bernard 1998). African Americans usually have higher B12 levels compared to whites (Stabler 1999).

Elevated levels of homocysteine are termed hyperhomocysteinemia. There is no agreed-upon cutpoint. Cutpoints range from 10 to 16 µmol/L (Evatt 2010; Kuo 2005; Koehler 1996). Hyperhomocysteinemia is common in older adults, with a prevalence of about 30%, defined as > 14 µmol/L (Selhub 1993).

1.2 FUNCTIONS OF B12

Vitamin B12 converts folate to its active form (Zimmerman 2001). Tissue stores of folate are "trapped" as inactive without vitamin B12. Then, B12 and folate work synergistically for many essential functions in the body. Vitamin B12 is fundamental for amino acid metabolism and optimum fat metabolism. B12 and folate are needed for the synthesis of nucleic acids and DNA. Finally, B12 is critical for the nervous system. Vitamin B12 is vital for the synthesis of myelin (the protective sheath surrounding nerves in the periphery, spinal cord, and brain), for the maintenance of nerve cells throughout the nervous system and spinal cord, and for the normal function of nerve cells, although the specific function for which vitamin B12 is needed is unclear (Zimmerman 2001).

1.2.1 Amino acid metabolism

Homocysteine is a toxic, sulfur containing amino acid (Schulz 2007). It is methylated to form methionine by the cobalamin and folate-dependent enzyme. Methionine is an essential amino acid. Homocysteine can also condense with serine to form cystathionine. Cystathionine is an intermediate in the synthesis of cysteine, a non-essential amino acid that has an important structural role in many proteins. Folate reduces homocysteine; however, only to a certain point if vitamin B12 levels are low (Varela-Moreiras 2009). Therefore, a deficiency in either folate or B12 results in elevated levels of homocysteine (Johnson 2010; Flicker 2006; Bjorkegren 2004).

Elevated homocysteine levels are associated with older age, male sex, white race, smoking, high coffee consumption, lack of physical activity, and poor renal function (Nygard 1998; Carmel 1999; Gonzalez 2007).

1.2.2 Fat metabolism

Methylmalonic acid (MMA) is converted to succinyl-coenzyme A using vitamin B12 as a cofactor (Snow 1999). They are intermediates in the citric acid cycle, which is part of the metabolic pathway that converts carbohydrates, fats, and proteins into carbon dioxide and water for useable energy. Elevated MMA is a highly sensitive marker of B12 deficiency (Stabler 1998).

1.3 CLINICAL SIGNS AND SYMPTOMS OF B12 DEFICIENCY

Megaloblastic anemia is the classic initial clinical sign of B12 deficiency (Carmel 2008). Symptoms include fatigue, weakness, shortness of breath, and decreased ability to concentrate (Zimmerman 2001). It is usually caused by a deficiency in vitamin B12 or folate. Red blood cells are larger than normal, which may cause low numbers of white blood cells and platelets. Reduced platelet production may result in abnormal bleeding, and a low white blood cell count may reduce immunity. Additional symptoms of B12 deficiency include anorexia, constipation, and weight loss. Patients with vitamin B12 deficiency may also have irritability, hostility, forgetfulness, confusion, poor memory, agitation, psychosis, depression, numbness and tingling in the hands and feet, sensory loss, unsteady movements, poor muscular coordination, and an unstable gait (Kernich 2006; Zimmerman 2001; Bernard 1998).

1.4 VITAMIN B12 INTAKE

1.4.1 Food sources

Vitamin B12 is found naturally in animal products (e.g., eggs, milk, fish, beef, poultry) (Zimmerman 2001). Vitamin B12 is sensitive to heat and substantial amounts of B12 can be lost when heated (e.g., milk boiled for 2 minutes loses 30% of its vitamin B12). Plant sources unless enriched do not contain vitamin B12.

1.4.2 Absorption of protein-bound B12

The protein-bound B12 enters the stomach where gastric acid and pepsin release vitamin B12 from dietary protein. Then, vitamin B12 binds to haptocorrin, an R protein, in the stomach which is found in gastric juice (Andres 2004). In the intestine, pancreatic enzymes free B12 and B12 binds to intrinsic factor, a glycoprotein secreted by parietal cells in the stomach. B12 is then absorbed in the terminal ileum. Once absorbed, B12 binds to transcobalamin II and is transported throughout the body to the tissues.

1.4.3 Supplemental vitamin B12

The crystalline form of vitamin B12 is found in supplements and fortified foods. Absorption from the crystalline form does not decrease with age (Allen 2009). Vitamin B12 supplements are recommended for those not getting enough vitamin B12 through the diet (e.g., vegetarians). There are a wide range of doses available (e.g., 5 μg to 5 mg). Fractional absorption decreases

as the oral dose is increased (Allen 2009). The ability to absorb B12 is limited by the capacity of intrinsic factor, a protein that binds to vitamin B12 and is needed for absorption (Carmel 2008). A dose finding trial in community-dwelling adults aged ≥ 70 years, with vitamin B12 deficiency found that 647-1032 µg/day is needed to normalize B12 levels (Eussen 2005). Supplemental B12 can be found in various forms (e.g., oral tablet, nasal spray, intramuscular injection).

There are no reports of toxicity in healthy adults, even at very high oral doses (Zimmerman 2001). Excess B12 is excreted in the urine or feces. B12 intramuscular injections are rarely associated with allergic reactions (Zimmerman 2001).

1.4.4 RDA for vitamin B12

The 2010 Dietary Guidelines for Americans recommend that the RDA for B12 in adults > 50 years is 2.4µg per day, the majority coming from fortified foods and supplements (USDA & USDHHS, 2010). There is no tolerable upper limit.

1.4.5 Storage in the body

Unlike other water-soluble vitamins, about 2-5mg of vitamin B12 is stored in the body, of which 80% is stored in the liver (Snow 1999). B12 is secreted in the bile and reabsorbed in the small intestine, in a process called enterohepatic reabsorption (Baik 1999). Thus, it can take up to 5 years to develop B12 deficiency even if a person develops severe malabsorption (Snow 1999).

1.5 CAUSES OF B12 DEFICIENCY

Causes of B12 deficiency include insufficient vitamin B12 intake, from either the diet (e.g., vegetarians) or supplements. The prevalence of malnutrition in older adults is 5-10% living independently and 30-60% among those hospitalized or in a nursing home (Brownie 2006). However, in older adults, the main cause is food cobalamin malabsorption (>50% of cases) (Andres 2004; Andres 2005; Allen 2009). Other causes include pernicious anemia, gastrointestinal disease, gastric surgery, and certain medications.

1.5.1 Atrophic gastritis

Atrophic gastritis is chronic inflammation of the stomach lining (gastric mucosal) (Park 2006). It is usually caused by Helicobacteur pylori bacteria. H. pylori bacteria is present in about 80% of those with severe food-cobalamin malabsorption and affects about 50% of those aged 60 years or older, and 50-70% of adults over 80 years (Carmel 2001; Allen 2009; Salles 2007). Atrophy in the stomach cells that secrete acid and digestive enzymes lead to impaired digestion and absorption of vitamin B12. Chronic atrophic gastritis affects 10-30% of older adults (Baik 1999). It primarily affects the antrum, the distal part of the stomach, and results in a loss of gastric acid (i.e., achlorhydria). It is likely that most older adults can still absorb vitamin B12 in the crystalline form, (i.e., from fortified foods or supplements), unless the gastric atrophy is so severe that intrinsic factor can no longer be produced (Allen 2009).

1.5.2 Pernicious anemia

Pernicious anemia affects 1-2% of older adults (Park 2008). It is caused by weakening of the stomach lining or an autoimmune disorder that affects the parietal cells, which make intrinsic factor. Thus, there is a lack of intrinsic factor, and B12 cannot effectively be absorbed. Patients with pernicious anemia have a decrease in red blood cells. A diagnosis is made if a patient is positive for antibodies to intrinsic factor or has a positive Schilling test (Andres 2004). Serum gastrin and pepsinogen I abnormalities are highly sensitive (>90%) but lack specificity. Pernicious anemia explains 15-30% of older adults with vitamin B12 deficiency (Andres 2004; Andres 2005). Pernicious anemia can develop rapidly (i.e., 1-3 years), and the mean age at diagnosis is 60 years (Baik 1999). The prevalence is higher in older women (2.7%) than in older men (1.4%) (Carmel 1996). Most patients with pernicious anemia are treated with B12 intramuscular injections; however, it is believed that high doses of oral supplementation (e.g., 1-5mg) can be absorbed (Kuzminski 1998).

1.5.3 Medications that cause B12 deficiency

Certain medications that suppress gastric acid (e.g., proton pump inhibitors (PPIs), H-2 blockers) can decrease B12 levels with prolonged use (Dharmarajan 2008). Dharmarajan et al. found most older adults (54%) take acid-suppressing medications, (26% PPIs and 28% H-2 blockers). Also, metformin, a hypoglycemic medication used by patients with diabetes, can cause B12 deficiency (Wile and Toth 2010). Metformin-induced B12 deficiency is especially important in older adults, as 27% of older adults (65 years or older) had diabetes in 2010 (CDC 2011). The exact mechanism of how metformin impairs B12 absorption is unknown, hypotheses include bacterial

overgrowth in the intestines or disruption in calcium-dependent uptake of B12 (Buvat 2004; Bauman 2000). In a recent study of patients with diabetes, those treated with metformin for more than 6 months had lower B12 levels, and higher MMA and homocysteine levels compared to those with no metformin exposure, and metformin use was associated with more severe peripheral neuropathy (Wile and Toth 2010). An estimated 30% of adults with chronic metformin use have B12 deficiency (Bell 2010).

1.5.4 Other causes of B12 deficiency

Gastric surgery, especially malabsorptive procedures (e.g., gastric bypass), can cause B12 deficiency (Kazemi 2010). Bariatric surgery is one of the fastest growing surgeries in the United States with gastric bypass being the most popular procedure (Davis 2006). The prevalence of vitamin B12 deficiency after gastric bypass was shown to be as high as 33% (Vargus-Ruiz 2008). Diseases affecting the gastrointestinal tract (e.g., Crohn's disease) may also cause B12 deficiency (Andres 2004). Liver disease can cause lower B12 levels, since the liver stores B12 and produces proteins essential for the transport of B12 to cells (Zimmerman 2001). Heavy alcohol consumption and chronic smoking can cause B12 deficiency by damaging both the stomach lining and liver.

1.6 DEFINITIONS OF LOW/DEFICIENT B12

There is no agreed-upon definition for B12 deficiency. Using serum B12 alone is neither sensitive or specific to determine a tissue deficiency (Stabler 1997). MMA and homocysteine

are highly sensitive markers for B12 deficiency (Stabler 1998; Carmel 2003; Savage 1994). Cystathionine and 2-methylcitrate are more sensitive than MMA and homocysteine for assessing poor renal function (Stabler 1997).

Many studies use only serum B12 to define low or deficient B12 levels, and they use different cutpoints for deficiency (e.g., 74-148 pmol/L) and low (e.g., 185 to 260 pmol/L); some use low serum B12 and high MMA (e.g., > 2 or 3 SD above the mean); and a few use high MMA alone (Park 2006; Carmel 2003; Carmel 1988; Baik 1999; Snow 1999; Clarke 2007; Penninx 2000). While there is still no agreed-upon cutpoint for low or deficient B12 levels, the most commonly used serum B12 cutpoints are 148 pmol/L for deficiency, which is the clinical cutpoint, and 260 pmol/L for low B12. The cutpoint of 260 pmol/L was suggested using data from the Framingham study (Lindenbaum 1994), based on a high prevalence (i.e., 27.9%) of older adults with elevated MMA (i.e, > 3 SD above mean) among those who had serum B12 levels below 260 pmol/L, and this cutpoint has since been adopted by other studies (Park 2006; Tucker 2005; Carmel 2003).

1.7 OUTCOMES ASSOCIATED WITH B12 DEFICIENCY AND HYPERHOMOCYSTEINEMIA

Vitamin B12 deficiency has been associated cross-sectionally with hematological disorders: megaloblastic anemia and pancytopenia, a deficiency in all blood cells; neuropsychological disorders: dementia, cognitive impairment, depression; cardiovascular events: increased risk of myocardial infarction and stroke (Andres 2004; Bernard 1998; Baik 1999; Johnson 2003; Oh 2003). Poor B12 may also cause hearing loss (Park 2006). Low B12 levels were significantly

associated with low broadband ultrasound attenuation, high markers of bone turnover, and increased fracture risk (Dhonukshe-Rutten 2005). The incidence and prevalence of the symptomatology of low vitamin B12 is poorly defined.

More than 25% of older adults have peripheral neuropathy or clinically positive tests for neuropathy (Baldereschi 2007; Rivner 2001; Resnick 2001; Gregg 2004). The prevalence of peripheral neuropathy is high in older adults, and increases with age. In the 1999-2000 NHANES, 28% of adults aged 70-79, and 35% of adults aged 80 years or older had peripheral neuropathy, based on self-reported symptoms or insensitivity to monofilament detection (Gregg 2004). Diabetes mellitus is a major risk factor for clinical peripheral neuropathy in older adults, yet only explains around 40% of prevalent cases and half of incident cases (Baldereschi 2007).

Most randomized clinical trials have not found a beneficial effect of B12 supplements. B vitamins were not associated with bone turnover (Green 2007). B12 vitamins had no effect on decreasing the incidence or severity of depression (Ford 2008). Vitamin B supplements did not reduce the risk for cardiovascular events (e.g., myocardial infarction, stroke, coronary revascularization, mortality from cardiovascular disease) (Carlsson 2006; Lonn 2006; Albert 2008) and had no effect on risk of total invasive cancer or breast cancer in women (Zhang 2008). However, many of these studies were underpowered (e.g., small sample size), had a short duration, and an insufficient dose of vitamin B12, compared to what Eussen et al. found in their dose-finding trial (Eussen 2005).

Elevated homocysteine levels are associated with coronary artery disease, myocardial infarction, stroke, vascular disease, thrombotic disease, poor renal function, cognitive impairment, dementia, depression, osteoporotic fractures, functional decline, and an increased risk of mortality (Carmel 1999; Kuo 2005; Gonzalez 2007). Though, a recent meta-analysis of

homocysteine lowering trials found no significant effects on cardiovascular events, cancer, or mortality (Clarke 2010).

1.8 VITAMIN B12, HOMOCYSTEINE AND PERIPHERAL NERVE FUNCTION

B12 deficiency is known to be associated with clinical peripheral neuropathy, myeloneuropathy, and optic neuropathy (Baik 1999; Klee 2000; Saperstein 2002; Volkov 2006). Subclinical associations with B12 deficiency include loss of senses in peripheral nerves and weakness in lower extremities (Mold 2004). In a randomized clinical trial, B vitamin supplements improved nerve conduction velocity in patients with diabetic polyneuropathy (Stracke 1996).

1.8.1 Implications for physical function and disability

Poor peripheral nerve function is associated with lower physical performance in older adults, independent of diabetes (Strotmeyer 2008). Reduced sensory and motor peripheral nerve function is related to lower quadriceps and ankle strength (Strotmeyer 2009) and lower bone mineral density (Strotmeyer 2006). Poor peripheral nerve function is also associated with slower gait speed, poor balance and falls (Resnick 2000; Deshpande 2008; Richardson 1992; Richardson 1995; Koski 1998; Sorock 1992; Luukinen 1995). In the MacArthur Study of Successful Aging, the relationship between vitamin B12, homocysteine, and physical performance in older adults was examined (Kado 2002). Results showed participants with elevated homocysteine had an increased risk of functional decline over 3-years. Lower vitamin B12 levels were associated with a greater 3-year decline in physical function. High

homocysteine has also been associated with slower gait speed and lower quadriceps strength (Rolita 2010; Soumare 2006; Kuo 2007).

1.9 VITAMIN B12 AND COGNITIVE FUNCTION

1.9.1 Biology

The physiologic pathway between vitamin B12 and cognition is not fully understood (Malouf 2003). Figure 1 illustrates hypothesized pathways between vitamin B12 and cognition. Vitamin B12 is essential to convert homocysteine to methionine which is then converted to S-adenosylmethionine [SAM] (de Lau 2009). SAM is required for normal brain function. Low B12 may lead to a deficiency in SAM, causing oxidative damage, fewer neurotransmitters (e.g., dopamine, serotonin, melatonin), and impaired cognition. Elevated homocysteine levels may be toxic to neurons, resulting in cognitive impairment, and can also cause vascular damage which may slow information processing speed leading to decreased cognitive function (Malouf 2009). Finally, B12 deficiency can cause damage to myelin as a result of deficient methylation of myelin basic protein (Weir 1999). Demyelination causes damage to the white matter which may slow information processing speed leading to cognitive impairment (Smith 2009).

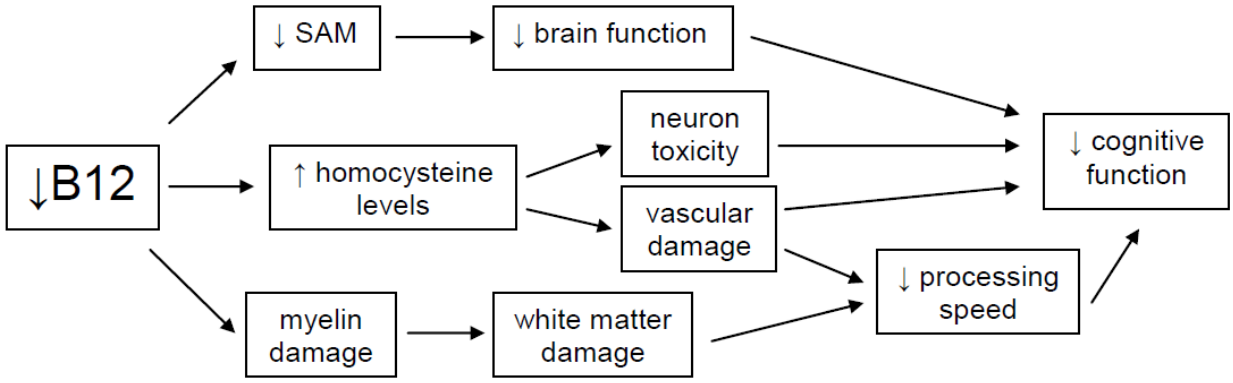

Figure 1. Hypothesized pathways to cognitive impairment

1.9.2 Observational Studies

Many studies have examined the relationship between vitamin B12 and cognition; however, the results have been inconsistent (Kang 2006). Some studies have found an association between B12 and cognition (Elias 2006; Johnson 2003; Goodwin 1983), while others have not (Kang 2006; Teunissen 2003). In cross-sectional studies of older adults, B12 was found to be associated with global cognitive function, as well as domains of executive function, perceptional reasoning, and construction [see Table 1] (Wahlin 2001; Riggs 1996). In older men from the Normative Aging Study, low B12 was associated with worse performance on the Spatial Copying Test which measures motor performance in addition to perceptual reasoning. Vitamin B12 deficiency was associated with lower Mini-Mental State Examination (MMSE) scores in a UK study of 84 older adults without dementia (McCracken 2006) and in the Banbury B12 Study (Hin 2006). However, MMSE assesses cognitive domains of orientation, registration, attention, recall, and language, not speed and motor performance to which B12 is hypothesized to be related.

In a 10-year longitudinal study of older adults (Oxford Healthy Aging Study), low vitamin B12 status (i.e., high homocysteine and high MMA levels) was significantly associated with lower MMSE scores at both baseline and follow-up and greater cognitive decline; however, serum B12 levels were not associated with MMSE scores cross-sectionally or longitudinally [Table 1] (Clarke 2007). Most longitudinal studies, including the MacArthur Study, the Leiden 85-Plus Study, and the Nurses' Health Study, have found no association between B12 and cognitive function or decline (Kado 2005; Mooijaart 2005; Kang 2006). These studies all analyzed B12 as a continuous predictor and did not use cutpoints for low or deficient B12. In the MacArthur Study and in the Nurses' Health Study, none of the cognitive tests assessed processing speed or motor performance. The Leiden 85-Plus Study included a test of processing speed (letter digit coding test); however, they excluded participants who had low MMSE scores, which may have biased the results.

1.9.3 Randomized Clinical Trials

In a randomized clinical trial of older adults with mild cognitive impairment, B vitamins [folic acid, B6, B12] slowed the rate of brain atrophy (Smith 2010). In another clinical trial, B12 supplementation improved cognitive and cerebral function in older B12 deficient adults; performance on the Verbal Word Leaning Test, Verbal Fluency and Similarities improved, which measure memory and executive function, and the quantitative electroencephalograph showed more fast activity and less slow activity (van Asselt 2001). However, Table 2 shows many randomized clinical trials found B vitamins do not improve cognitive performance (McMahon 2006; Stott 2005; Eussen 2006), or slow the rate of cognitive decline (Aisen 2008). Possible reasons for null findings are the clinical trials were underpowered, too short in duration,

gave an insufficient dose of B12 to reach a therapeutic level, included subjects with normal vitamin B12 levels or no cognitive impairment, or the tests assessed cognitive domains other than processing speed. In addition, most of these studies looked for an improvement in cognition rather than delaying or slowing cognitive decline.

1.10 PUBLIC HEALTH IMPLICATIONS

1.10.1 Aftermath of folic acid fortification

Folic acid is the form of folate which is added to foods and supplements. The United States mandated the addition of folic acid to enriched breads, cereals, flours, corn means, pasta, rice and other grain products by January, 1998. Canada, Chile, and Australia also have mandatory folic acid fortification (Oakley 2010). While the fortification has shown benefits, improving folate and homocysteine levels in the population as a whole (Jacques 1999), large doses of folic acid in a B12 deficient person may cause megaloblastic anemia to remit (Baik 1999; Varela-Moreiras 2009). This is referred to as "masking of anemia". Anemia is often the first sign of B12 deficiency. Thus, older adults with B12 deficiency may lack anemia, while symptoms of neurological impairments progress (Baik 1999). The longer the delay before treatment, the less likely neurological symptoms can be reversed (Varela-Moreiras 2009). The Flour Fortification Initiative recommends vitamin B12 fortification because consuming large amounts of folic acid may delay the diagnosis of B12 deficiency in older adults (Allen 2010). In the aftermath of folic acid fortification, B12 deficiency is now the primary modifiable risk factor of hyperhomocysteinemia, above folic acid deficiency (Liaugaudas 2001; Johnson 2003).

Furthermore, it is important to assess B12 levels and neurological function in the aftermath of the folic acid fortification.

1.10.2 Importance of studying vitamin B12 and neurological function in older adults

There is a high prevalence of older adults with low or deficient B12 levels. B12 deficiency is a known cause of clinical peripheral neuropathy; however, little is known about how low B12 levels affect peripheral nerve function. Both cross-sectional and longitudinal studies are lacking. Implications for disability associated with subclinical peripheral nerve function exist and identifying key risk factors for poor peripheral nerve function is critical. The relationship between vitamin B12 and cognition is unclear; however, few studies have examined the relationship between vitamin B12 and processing speed, the cognitive domain most likely to be affected by B12 deficiency. Importantly, critical levels have not been determined for serum B12 levels related to higher neurological function.

Table 1. Observational Studies of Vitamin B12 and Cognitive Function

Author (year)	Design	Study Population	Cognitive Test	B12 Assessment	Results
Hin (2006)	Cross-sectional	1000 community-dwelling adults aged 75+ years from the Banbury B12 Study; 60% female	MMSE	B12 (cutpoint: 133 pmol/L)	**Deficient B12 was associated with lower MMSE scores.**
McCracken (2006)	Cross-sectional	84 older adults in UK without dementia (aged > 69 years); 50% female	MMSE	B12, holoTC, MMA (continuous)	**Higher MMA** (but not B12 or holoTC) **was associated with lower MMSE scores.**
Clarke (2007)	Longitudinal	1648 community-dwelling older adults (≥65 years) in the Oxford Healthy Aging Study; 59% female	MMSE	B12, holoTC, MMA, Hcy (continuous)	**Low holoTC, high Hcy, and high MMA were associated with baseline cognitive function and greater 10-year cognitive decline.** B12 was not associated with MMSE scores at baseline and after 10 years.
Moojaart (2005)	Longitudinal	599 adults aged 85 years from the Leiden 85-Plus Study; 66% female	MMSE, Stroop, Letter Digit Coding Test, Word Recall Tests	B12 (continuous)	B12 was not associated with cognitive function or cognitive decline.
Riggs (1996)	Cross-sectional	70 older men (aged 52-81 years) from the Normative Aging Study	Verbal Fluency, Boston Naming Test, Vocabulary, Pattern Comparison, Continuous Performance Test, Word List Memory Test, Backward Digit Span, Activity Memory, Pattern Memory, Spatial Copying Test, Spatial Reasoning Test	B12 (continuous)	**Low B12 levels were associated with poorer scores on the spatial copying test.**
Kado (2005)	Longitudinal	499 high-functioning community-dwelling adults aged 70-79 years (54% female) at baseline from the MacArthur Study	Confrontation naming, Delayed Recognition Span Test, Similarities test	B12 (continuous)	No association was found between B12 and cognitive function or 7-year cognitive decline.
Kang (2006)	Longitudinal	635 women aged 70+ at baseline from the Nurses' Health Study (391 with follow-up data)	Telephone Interview for Cognitive Status, East Boston Memory Test	B12 (continuous)	B12 was not associated with cognitive function or cognitive decline.

MMSE=Mini-Mental State Examination; holoTC=holotranscobalamin; MMA=methylmalonic acid; Hcy=homocysteine

Table 2. Clinical Trials of B12 Supplementation and Cognitive Function

Author (year)	Study Population	Cognitive Test	B12 Supplementation	Results
Van Asselt (2001)	16 older adults aged 64-89 years with deficient B12 (≤150 pmol/L)	MMSE, Trail Making Test, Similarities, Rivermead Behavioral Face Recognition Test, Verbal Word Learning Test, Verbal Fluency, Forward and Backward Digit Span	5 months of intramuscular injections	**B12 supplementation improved performance on the Verbal Word Learning Test, Similarities, and Verbal Fluency test.**
McMahon (2006)	276 adults aged 65+ years with hcy>13 μmol/L	MMSE, Wechsler Paragraph Recall, Category Word Fluency, Rey Auditory Verbal Learning Test, Raven's Progressive Matrices, Controlled Oral Word Association, Trail Making Test B	2 years of folic acid, B6, and B12 supplementation	Vitamin B supplementation did not improve cognitive performance.
Stott (2005)	185 adults aged 65+ years with ischemic vascular disease and excluded if low B12 (<185 pmol/L) or major cognitive impairment (MMSE<19)	Letter Digit Coding Test, Telephone Interview for Cognitive Status	12 weeks of folic acid, B6, and B12 supplementation	Vitamin B supplementation did not improve cognitive performance.
Eussen (2006)	195 adults aged 70+ years with B12 levels between 100-200 pmol/L or B12 between 200-300 pmol/L MMA≥0.32 μmol/L, & creatinine≤120 μmol/L	Finger tapping, Motor planning, Figure of Rey, 15 Word Learning Test, Trail Making Test, Digit Span Forward and Backward, Raven, Stroop Test, Similarities, Word Fluency	24 weeks of B12 supplementation only or B12 and folic acid	Vitamin B supplementation did not significantly improve scores on any cognitive test.
Aisen (2008)	409 adults with mild to moderate AD (MMSE 14-26) and normal B12, folate, and homocysteine levels	MMSE, Alzheimer Disease Assessment Scale – cognitive subscale, Clinical Dementia Rating sum of boxes	18 months of folic acid, B6, and B12 supplementation	Vitamin B supplementation did not slow the rate of cognitive decline.

MMSE=Mini-Mental State Examination; MMA=methylmalonic acid; Hcy=homocysteine; AD=Alzheimer's Disease

2.0 VITAMIN B12 AND HOMOCYSTEINE LEVELS AND 6-YEAR CHANGE IN PERIPHERAL NERVE FUNCTION AND NEUROLOGICAL SIGNS

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2.1 ABSTRACT

Objective – To determine whether changes in vitamin B12 or homocysteine levels are associated with longitudinal changes in peripheral nerve function and clinical neurological signs and symptoms.

Methods – Participants aged ≥60 years (n=678; 72.2 ± 6.2 years at baseline; 43.5% male) were from the InCHIANTI Study. Low B12 (<260 pmol/L) and high homocysteine [Hcy] (≥13 µmol/L) were measured at baseline and 3-year follow-up. Neurological function was assessed by peroneal nerve conduction amplitude [CMAP] and velocity [NCV], neurological examination, and symptoms of peripheral neuropathy at baseline, 3-year and 6-year follow-up.

Results – At baseline, 43.8% had low B12 levels and 58.6% had high homocysteine levels. Over 6-years, 12.4% declined from normal to poor CMAP [<1mV] and 42.1% declined from normal to poor NCV [<40m/s]. In mixed models analyses, high homocysteine at both times was associated with worse CMAP at each visit compared to normal Hcy at both times (p=.04), adjusting for age, sex, site, diabetes (DM), and folate level. Participants who had normal Hcy levels at baseline and high at 3-year follow up were more likely to become unable to detect monofilament at 6-year follow-up compared to those with normal Hcy levels at both times (OR: 5.4; 95% CI: [1.5,19.0]), after adjusting for age, sex, site, DM, BMI, and PAD.

Conclusions – High homocysteine may be associated with worse sensory and motor peripheral nerve function. Since poor peripheral nerve function has been associated with lower

strength and physical performance in older adults, these results have important implications for disability in older adults.

2.2 INTRODUCTION

Vitamin B12 (cobalamin) deficiency affects 5-20% of older adults (Baik 1999; Andres 2004). Up to 40% have low vitamin B12 levels, though the health implications of low vs. deficient levels are not clear (Baik 1999; Park 2006; Varela-Moreiras 2009). The prevalence of low or deficient vitamin B12 levels increases with age (Bernard 1998). B12 deficiency can cause damage to myelin as a result of deficient methylation of myelin basic protein (Weir 1999). Along with hematological disorders, neuropsychological disorders, cardiovascular diseases, vitamin B12 deficiency has been associated with myeloneuropathy, optic neuropathy, peripheral neuropathy, loss of sensation in peripheral nerves, and weakness in lower extremities (Baik 1999; Klee 2000; Saperstein 2002; Volkov 2006).

Since, vitamin B6, B12 and folate work synergistically to convert homocysteine [Hcy] to methionine, low B12 or folate levels may result in high Hcy levels. Hyperhomocysteinemia, elevated Hcy levels, has a prevalence of about 30% in older adults (Selhub 1993). Elevated Hcy levels may be toxic to neurons and cause vascular damage (Malouf 2003). Hyperhomocysteinemia has been associated with older age, male sex, white race, smoking, high coffee consumption, lack of physical activity, cognitive impairment, depression, cardiovascular disease, poor renal function, functional decline, and an increased risk of mortality (Nygard 1998; Carmel 1999; Carmel 2003; Kuo 2005; Gonzalez 2007). High homocysteine has been shown to be related to slower gait speed, lower quadriceps strength, and greater decline in physical

function, which has clear implications for disability in older adults (Kado 2002; Rolita 2010; Soumare 2006; Kuo 2007).

The prevalence of poor peripheral nerve function and neuropathy is high in older adults and increases with age, >25% of older adults have peripheral neuropathy or clinically positive tests for neuropathy (Baldereschi 2007; Rivner 2001; Resnick 2001; Gregg 2004). Diabetes mellitus is the major risk factor for clinical peripheral neuropathy in older adults but only accounts for about half of those with clinical peripheral neuropathy (Baldereshi 2007). Subclinically, poor peripheral nerve function is associated with lower physical performance and lower strength in older adults, independent of diabetes mellitus (Strotmeyer 2008; Strotmeyer 2009). Therefore, implications for disability associated with subclinical peripheral nerve function exist and identifying key risk factors for poor peripheral nerve function is critical.

To our knowledge, there have been no epidemiological cohort studies examining the association between vitamin B12 or homocysteine with peripheral nerve function. In addition, there have been no previous studies which have studied the longitudinal changes in vitamin B12 or homocysteine levels in relation to changes in peripheral nerve function in older adults, despite the clear association of both with aging. We hypothesized that low B12 and high homocysteine levels and sustained low B12 and high homocysteine levels will be associated with greater decline in neurological function, compared to those with sustained normal B12 and homocysteine levels. The purpose of this study is to examine whether low vitamin B12 levels or high homocysteine levels are associated cross-sectionally or longitudinally with: 1) motor peripheral nerve function, 2) clinical neurological signs, and 3) symptoms related to peripheral neuropathy.

2.3 METHODS

2.3.1 Study population

Participants were from the InCHIANTI Study, a population-based study in the Chianti region of Italy. The InCHIANTI Study is an ongoing, longitudinal cohort study examining factors contributing to the decline of mobility in late life. Details of the study have been described elsewhere (Ferrucci 2000). Briefly, in August 1998, 1616 adults, aged 21-102 years, were selected from the population registry of Greve in Chianti (a rural area; 11,709 inhabitants, with 19.3% of the population 65 years or older) and Bagno a Ripoli (Antella village, near Florence; 4,704 inhabitants, with 20.3% of the population 65 years or older). The participation rate was 90% (1453 of 1616). We included 678 participants (56% of baseline) who were 60 years or older at the baseline visit with vitamin B12 or homocysteine levels measured at baseline and 3-year follow-up and motor peripheral nerve function, a neurological examination, or symptoms related to peripheral neuropathy assessed at baseline, 3-year, and 6-year follow-up.

There were 1203 participants who were 60 years or older at baseline, and 1091 (91%) had vitamin B12 or homocysteine levels measured. All 1091 participants had data for at least one neurological measure. At the 3-year follow-up, 70 refused to participate, 10 moved away from the area, 3 could not be located, and 107 were deceased. Of the 892 participants who had a 3-year follow-up visit, 797 participants (89%) had vitamin B12 or homocysteine measured. Of those, 119 did not have a 6-year follow-up visit (18 refused to participate, 6 moved away from the area, and 95 participants had died), leaving 678 participants in our analytic sample. The study was approved by the Ethics Committee of the Italian National Institute of Research and Care of Aging, and informed consent was obtained from all participants.

2.3.2 Assays

Serum samples were obtained by collecting blood in evacuated tubes without anticoagulant and stored at -80˚C. Vitamin B12 was measured with a radioligand-binding assay (SimulTrac-SNB Radioassay; ICN Pharmaceuticals). The minimum detectable concentration was 75 ng/L and the intraassay and interassay CVs were 11.0% and 12.0%, respectively. Plasma homocysteine concentrations were measured by a fluorimetric polarized immunoassay method (IMX; Abbott Laboratories) (Gori 2005). The sensitivity of the IMX Hcy assay was 0.5 mmol/L, and the interassay CV was 4.1%.

Serum folate, vitamin B6, and total cholesterol, creatinine, and inflammatory markers (IL-6 and TNF-α) were measured with commercial assays, which were previously described (Gori 2005; Gori 2006).

Low vitamin B12 was defined as <260 pmol/L and normal B12 as ≥260 pmol/L (Tucker 2005), and high homocysteine was defined as ≥13 μmol/L and normal Hcy as <13 μmol/L (Jacques 1999). Vitamin B12 and homocysteine were measured at the baseline visit and at 3-year follow-up. Categories of change from baseline to 3-year follow-up included: 1) Stay Normal; 2) Poor to Normal; 3) Normal to Poor; 4) Stay Poor.

2.3.3 Peripheral motor nerve function

Standard surface electroneurographic (ENG) studies of the right peroneal nerve were conducted within 3 weeks of the home interview by a trained geriatrician (Ferrucci, 2000). All studies were performed on an ENG-neuro MYTO device (E.B. Neuro S.p.A, Florence, Italy) using standard ENG-neuro disposable electrodes. A detailed description of the methods is reported elsewhere

(Lauretani, 2006). Nerve conduction amplitude in millivolts (compound motor action potential [CMAP]) and velocity in meters per second (nerve conduction velocity [NCV]) were measured between the fibular head and ankle. Poor CMAP was defined as <1 mV and poor NCV was defined as <40 m/s, according to clinical norms.

2.3.4 Neurological examination

Neurological signs within the motor system, sensory system, and cranial nerves were assessed in the examination. Details of the neurological examination in the InCHIANTI Study were previously described (Ferrucci 2004). The protocol for the neurological examination was designed to minimize subjectivity. The intraclass correlation coefficient for test-retest reliability for each item was > 0.8.

Neurological signs evaluated in relationship to low vitamin B12 were 1) abnormal touch sensitivity, 2) abnormal vibration sensitivity, 3) impaired sense of ankle position, 4) abnormal deep tendon reflexes, 5) positive Babinski reflex, and 6) a positive Romberg sign. Participants were excluded from the neurological exam analyses if they had Parkinson's disease, history of stroke, or multiple sclerosis.

2.3.5 Symptoms related to peripheral neuropathy

Participants were asked 1) "at present, do you ever experience pain in the left foot? …right foot?" 2) "at present, do you ever experience a sensation of coldness in the left foot? … right foot?" These symptoms were assessed at baseline, 3 and 6-year follow-ups.

2.3.6 Potential confounders

Potential confounders were identified if they were associated with vitamin B12 or homocysteine and neurological function and were included as covariates in the multivariable analyses. Demographic and health information were assessed at the baseline visit. Diabetes mellitus (DM) was diagnosed using the criteria from the American Diabetes Association (Expert Committee 2003). The presence of chronic health conditions (e.g., hypertension, peripheral arterial disease, congestive heart failure, myocardial infarction, stroke, thyroid disease) was determined using an adjudication process that included self-reported history, physical examination (e.g., blood pressure), and medical records information (Ferrucci 2000). Smoking status was assessed by self-report. Alcohol intake (g/day) was estimated by administration of the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire (Pisani 1997). To assess physical activity (PA), participants were asked if they performed any sport or recreational activity for at least 3 months in the last year (Elosua 2005). If yes, they were asked to specify the type and frequency and PA was then categorized as 1) sedentary (hardly any PA; mostly sitting); 2) light (light exercise 2-4 hrs/week); and 3) moderate-high (moderate or intense exercise). Body mass index (BMI) was calculated as weight (kg)/ height^2 (m^2).

2.3.7 Statistical Analyses

Differences in demographics, lifestyle factors, body composition, chronic health conditions, and cardiovascular risk factors were tested by change in vitamin B12 level and change in homocysteine level, using Pearson χ^2 tests or Fisher's exact test for categorical variables and ANOVA, Kruskal-Wallis or t-tests for continuous variables.

Multivariable logistic regression was performed at baseline for abnormalities from the neurological exam and for symptoms related to sensory neuropathy. Abnormalities from the neurological exam were modeled individually for each component described in the methods. The symptoms related to neuropathy were 1) numbness in either foot (y/n) and 2) coldness in either foot (y/n). Multinomial logistic regression was used to model change in signs or symptoms, using change in B12 levels and change in Hcy levels, separately as independent variables.

For nerve conduction, cross-sectional analyses was performed at baseline, using separate multivariable linear regression models for each independent variable (baseline low B12 and high homocysteine) with each outcome for 1) baseline CMAP and 2) baseline NCV. Multivariable linear regression was used for longitudinal change, separately for each predictor (change in vitamin B12 level and change in homocysteine level) for outcomes of 1) 6-year change in CMAP and 2) 6-year change in NCV. Mixed linear modeling was performed separately for each motor nerve function variable (using combined data from baseline, 3-year and 6-year follow-up) with change in vitamin B12 level and change in homocysteine level modeled individually as the independent variables.

The models were built progressively in order, adjusting for potential confounders: demographics (age, sex, clinic site), diabetes, BMI, lifestyle factors (smoking status, alcohol intake, activity level), cardiovascular risk factors (hypertension, CVD, PAD, MI, stroke), thyroid disease, use of hypoglycemic medications, inflammatory markers (IL-6, TNF-α), and folate and vitamin B6 levels. Mini-mental state examination (MMSE) was additionally included for the neurological exam abnormalities and the self-reported symptoms. Age, gender, clinic site, and diabetes were included in all models; other variables were removed if $p > 0.10$. Sensitivity

analysis was conducted removing participants with diabetes, and excluding from analysis those who changed categories for B12 or Hcy but only had a small amount of overall change (e.g., B12 within 50 pmol/L; Hcy within 1 μmol/L). Multicollinearity for independent variables was assessed using the variance inflation factor (VIF); no VIF was >2. All analyses were conducted with SAS, version 9.2 (SAS Institute Inc, Cary, NC).

Comparing participants who only had baseline data to those with baseline and 6-year follow-up, those with baseline only data were more likely to be older (77.7 ± 7.7 years vs. 72.2 ± 6.2 years; p=<.0001) and have diabetes mellitus (15.3% vs. 9.0%; p=.006). Participants with only baseline data had a significantly higher prevalence of high homocysteine (73.6% vs. 56.4%; p<.0001) at baseline compared to those with 6-year follow-up, and also worse CMAP (5.1 ± 3.1 mV vs. 6.8 ± 3.2mV; p=<.0001) and NCV (43.4 ± 3.9 m/s vs. 44.5 ± 4.0 m/s; p=.0002).

2.4 RESULTS

Baseline characteristics of the InCHIANTI participants by change in B12 level and change in Hcy level are shown in Table 3 and 4, respectively. Men were more likely to have low B12 levels at follow-up. Higher alcohol consumption was associated with having low B12 at either or both times compared with having normal B12 at both times. Participants who had low B12 at both times had lower cholesterol levels compared with having normal B12 at either time. Participants with low B12 at baseline had lower folate levels compared to having sustained normal B12. Sustained normal B12 levels were associated with lower homocysteine levels at baseline compared to having low B12 at either time. High homocysteine was associated with older age, male sex, higher alcohol consumption, higher IL-6 and TNF-α levels, lower B6, B12,

and folate levels. Participants who had high Hcy at both times were more likely to have hypertension compared to those who had sustained normal levels or who improved to normal levels.

The prevalence of low B12 decreased slightly from baseline (44%) to 3-year follow-up (39%) while the prevalence of high Hcy decreased considerably from 59% to 46% at follow-up. More than half of the participants had low B12 at one time (53%) with 30% having low levels at both times. About 41% had high homocysteine at both times, only 37% had normal Hcy at both times, and 18% improved to normal at the 3-year follow-up visit.

Table 5 shows baseline motor nerve function by baseline vitamin B12 and Hcy. Cross-sectionally, participants with high homocysteine had significantly lower nerve conduction velocity at baseline (β=-.71; p=.04), but after adjusting for potential confounders, the association was no longer significant (β=.40; p=.22). Sex attenuated the association.

Table 6 illustrates motor nerve function by change in B12 levels though no significant differences existed between groups. Table 7 reports motor nerve function by change in Hcy levels. Those who had high Hcy at both times or became high at follow-up had significantly lower NCV compared to those with sustained normal levels. The prevalence of poor CMAP [<1 mV] and NCV [<40 m/s] increased at each time point, with a substantial increase in poor NCV at the 6-year follow-up, with more than 50% having poor NCV.

About 12% of the participants declined to poor CMAP. There were only 7 participants who improved to normal amplitude (mean increase of 1.6 ± 1.1 mV), and those who had poor levels at both baseline and follow-up had very little decline (-0.2 mV) since they already had low levels (<1 mV). Less than half (49%) had normal NCV at both baseline and 6-year follow-up. There were 42% who had normal NCV at baseline and declined to poor NCV at follow-up (mean

decline: 7.1 ± 3.3 m/s). Eight percent of participants had poor NCV at baseline and follow-up, and they had the least amount of change (-2.4 m/s).

In the multivariable mixed models, no significant association was found between changes in B12 and changes in CMAP or NCV. However, for homocysteine, high Hcy at baseline and 3-year follow-up was associated with worse CMAP across visits compared to sustained normal levels of Hcy (p=.04), after adjusting for age, sex, site, diabetes, and folate level [Table 8]. Change in homocysteine was not significantly associated with NCV changes after adjustments or NCV longitudinal trend, sex and diabetes attenuated the association.

There was no association between B12 or homocysteine and symptoms of numbness and/or coldness in either foot, cross-sectionally or longitudinally, after adjusting for covariates.

At baseline, high homocysteine was not associated with abnormal touch sensitivity (monofilament) after adjusting for covariates. BMI and physical activity attenuated the association. In the longitudinal analysis, participants whose homocysteine levels were normal at baseline and high at 3-year follow up were more likely to become unable to detect monofilament at 6-year follow-up compared to those with normal homocysteine levels at both times (OR: 5.4; 95% CI: [1.5,19.0]), after adjusting for age, sex, site, DM, BMI, and PAD.

We performed sensitivity analysis to remove participants from the analysis who changed categories for B12 or Hcy but only had a small amount of change, and also removing participants with diabetes, and the results were consistent.

2.5 DISCUSSION

Our results show that high homocysteine was associated with poor motor and sensory peripheral nerve function (CMAP and inability to detect 4g monofilament touch). These findings are important because B12 deficiency is primarily associated with sensory neuropathy (Smith 2004) and worse nerve function may lead to impaired physical function and disability in older adults (Resnick 2000; Resnick 2001; Strotmeyer 2008). This is the first study, to our knowledge, that has examined the association between homocysteine and peripheral nerve function, cross-sectionally or longitudinally, and that considered change in B12 or Hcy and change in peripheral nerve function.

We found no association between B12 or Hcy and symptoms related to clinical peripheral neuropathy, cross-sectionally at baseline or longitudinally. The symptoms may have been too general and the symptoms may have been caused by other factors (e.g., other diseases or injuries). Also, the participants were asked "at present" whether they had the symptoms. Symptoms may only be present early in peripheral neuropathy and often peripheral neuropathy is asymptomatic (Franse 2000). In addition, certain symptoms associated with peripheral neuropathy such as deep aching or burning pain were not assessed. Also, none of the neurological signs examined were associated with low B12 levels. This is an important finding, because older adults may not have clinically recognized neurological signs or symptoms of poor B12 levels and thus low or deficient B12 levels may not be detected. Additionally, these neurological signs may be associated with severe B12 deficiency.

There was a very high prevalence of low B12 and high homocysteine levels, which was higher than previous studies found (Baik 1999; Selhub 1993). In Italy, there is no mandatory folic acid fortification which there has been in the U.S. since 1998 (Pfeiffer 2008). Since folic

acid can lower homocysteine levels, we expected a higher prevalence of elevated homocysteine levels compared to the United States. Also, it is important to note that alcohol consumption is common in Italian older adults (Buja 2010), and is related to higher levels of Hcy (Cravo 2000; Jacques 2001). However, alcohol consumption has also been shown to have a J-shaped association, where moderate consumption is associated with a lower Hcy concentration and high consumption is associated with high homocysteine levels (Halsted 2001). The high prevalence of poor B12 and Hcy levels may also be attributed to how we defined low B12 and high Hcy, with a higher B12 cutpoint and a lower Hcy cutpoint. We used these cutpoints, because while vitamin B12 deficiency is a known cause of peripheral neuropathy, little is known about how low B12 levels or moderate hyperhomocysteinemia levels affect peripheral nerve function in older adults, and prevalences of both low B12 and elevated Hcy are high among older adults. Finally, high prevalences of low B12 and elevated Hcy, may also be due to a very low prevalence of any supplemental vitamin use in our study population. At baseline, less than 4% took any supplements (Bartali 2006).

In our study population, the prevalence of high Hcy decreased from baseline to 3-year follow-up. Lifestyle factors such as smoking, low physical activity, and excessive coffee and alcohol consumption can cause higher homocysteine levels (Nygard 1998; El Khairy 1999; De Bree 2001). Thus, decreasing coffee or alcohol consumption, increasing physical activity, vitamin B6, B12 or folic acid supplementation, and smoking cessation can reduce homocysteine levels. Participants and their physicians also received the test results, and they may have subsequently changed their diet or lifestyle habits or started folic acid, vitamin B6, or vitamin B12 supplements.

The prevalence of poor CMAP and NCV was also high and increased at each time point, with a considerable increase in poor NCV at 6-year follow-up. The prevalence of poor nerve function increases with age. Nerve function declines as age increases (Baldereschi 2007). In the U.S. for 1999-2000, 28% of adults aged 70-79 years and 35% of adults aged ≥80 years had peripheral neuropathy based on a simple screen for reduced sensation at the foot (Gregg 2004). Low CMAP is related to nerve axonal damage and low NCV is related to nerve demyelination, and we expected NCV to decline before CMAP since demyelination is thought to occur before axonal degeneration because myelin covers and protects the axons (Arezzo 2002).

One limitation of this study was having a small number of participants who declined from normal to poor B12 and Hcy. Also, vitamin B12 and homocysteine levels were not tested at the 6-year follow-up, and methylmalonic acid testing was not done. Serum B12 may not be the best marker to determine low B12 levels (Savage 1994). There was no objective sensory nerve function assessed from the nerve conduction test. Importantly, our study included older adults who lived in Italy, and our findings may not be generalizeable to other populations, due to differences in diet and supplement practices and folic-acid fortification. However, it is critical to determine the association between B12 and Hcy and nerve function in a non-folic acid fortified country.

Since participants with only baseline data had higher homocysteine levels and worse nerve function at baseline, the models may underestimate the effect of the association if the dropouts were more likely to have progression of peripheral neuropathy. In addition, those who had poor CMAP or NCV at baseline did not have much room to decline since they already had very low levels. Thus, looking at continuous change in nerve function may be inappropriate for those who already had poor nerve function at baseline and cannot decline further.

A strength of this analysis was that it was longitudinal and included 3 timepoints for the outcomes. The longitudinal analysis was critical because we did not find any cross-sectional association, and we saw the impact of sustained high homocysteine levels on peripheral nerve function. We had a large cohort of older men and women. Measuring motor nerve function with nerve conduction is highly sensitive and reproducible and considered state of the art (England 2005). We had measures of both motor nerve function and sensory nerve function. Monofilament detection, while not as sensitive, it is very specific and has high clinical significance, because it is an inexpensive and quick test that can be done in an exam room (Armstrong 1998; Feng 2009). Previous work has shown high creatinine is associated with high homocysteine levels (Jacques 2001); however, we adjusted for creatinine in our models and found no difference.

This analysis shows that high homocysteine levels may be related to worse sensory and motor nerve function in older adults. These results have important implications for motor functioning and disability in older adults. Several researchers have identified a link between peripheral neuropathy and impaired balance and falls (Richardson 2002; Richardson 1995; Richardson 1992; Koski 1998; Luukinen 1995; Sorock 1992). B12, folic acid, and B6 can lower homocysteine levels and supplemental vitamins are readily available, adequately absorbed, and highly tolerated in older adults (McMahon 2006; Andres 2010).

2.6 TABLES

Table 3. Baseline descriptive characteristics by change in vitamin B12 levels

	Stay Normal (n=313)	Low to Normal (n=89)	Normal to Low (n=60)	Stay Low (n=202)	p-value
Age (years)	71.7 ± 5.9	72.4 ± 7.1	71.8 ± 5.6	73.1 ± 6.4[c]	0.19
Male (%)	33.6	43.8	56.7[b]	54.5[c]	<0.0001
Diabetes mellitus (%) Hypoglycemic medication use (%)	15.0 47.4	5.6[a] 87.5	14.3 40.0	9.6 58.7	0.06 0.22
Smoking status Former (%) Current (%)	 24.6 12.8	 25.8 16.9	 23.3 13.3	 29.7 18.3	0.33 0.58 0.35
Alcohol use (g/day)	11.5 ± 16.2	17.3 ± 21.9[a]	19.0 ± 19.1[b]	20.8 ± 27.1[c]	0.0002
Physical activity level Sedentary (%) Light (%)	 15.1 45.3	 14.6 52.8	 18.3 43.3	 11.4 43.8	0.47 0.51 0.52
BMI (kg/m^2)	27.8 ± 4.2	27.6 ± 4.1	27.4 ± 3.3	27.6 ± 4.2	0.92
Height (cm)	158.1 ± 9.2	159.7 ± 10.0	161.2 ± 9.5[b]	160.6 ± 9.3[c]	.01
Weight (kg)	69.4 ± 11.9	70.4 ± 12.7	71.1 ± 11.1	71.3 ± 12.5	.36
Hypertension (%)	62.0	71.4	60.0	63.3	0.41
SBP (mmHg)	149.7 ± 19.5	149.8 ± 17.6	148.6 ± 19.5	148.6 ± 18.3	0.97
DBP (mmHg)	83.3 ± 8.5	85.1 ± 7.8	83.1 ± 8.4	85.2 ± 8.6[c]	0.03
PAD (%)	6.3	11.0	7.3	6.4	0.50
CHF (%)	1.7	4.4	7.6[b]	2.8	0.10
MI (%)	3.5	5.6	5.0	4.0	0.72
Stroke (%)	4.2	1.1	5.2	1.5	0.16
Thyroid disease (%)	9.7	4.6	6.7	5.5	0.22
Total cholesterol (mg/dL)	223.2 ± 40.4	227.3 ± 34.4	227.2 ± 38.2	212.1 ± 37.7[c,e,f]	0.005
Creatinine (mg/dL)	0.40 ± 0.50	0.34 ± 0.50	0.29 ± 0.46	0.35 ± 0.48	0.31
IL-6 (pg/mL)	3.2 ± 2.1	3.2 ± 2.3	3.3 ± 2.0	3.6 ± 2.5	0.57
TNF-α (pg/mL)	4.8 ± 3.0	4.6 ± 2.0	4.7 ± 2.5	5.1 ± 3.9	0.81
Folate level (nmol/L)	8.1 ± 4.8	6.6 ± 3.6[a]	7.8 ± 3.8	6.8 ± 3.4[c]	0.0006
Vitamin B6 level (nmol/L)	32.5 ± 27.3	28.3 ± 14.2	28.7 ± 15.7	25.4 ± 13.1[c]	0.06
Hcy level (µmol/L)	13.7 ± 3.9	15.0 ± 5.3[a]	14.9 ± 3.9[b]	16.9 ± 7.8[c,e]	<0.0001
Vitamin B12 level (pmol/L)	480.2 ± 295.2	204.1 ± 40.2[a]	348.6 ± 147.6[b,d]	177.7 ± 53.2[c,e,f]	<0.0001
Change in B12 (pmol/L)	8.5 ± 320.0	148.6 ± 170.2 [a]	-135.9 ± 153.5 [b,d]	-1.4 ± 51.3 [e,f]	<0.0001

Pairwise: p<0.05 for a: Low to Normal vs. Stay Normal; b: Normal to Low vs. Stay Normal; c: Stay Low vs. Stay Normal; d: Normal to Low vs. Low to Normal; e: Stay Low vs. Low to Normal; f: Stay Low vs. Normal to Low
Low B12: <260 pmol/L; Normal B12: ≥260 pmol/L

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PAD: peripheral arterial disease; CHF: congestive heart failure; MI: myocardial infarction

Table 4. Baseline descriptive characteristics by change in homocysteine levels

	Stay Normal (n=245)	High to Normal (n=119)	Normal to High (n=32)	Stay High (n=273)	p-value
Age (years)	70.1 ± 5.2	71.5 ± 6.1[a]	73.6 ± 6.3[b]	74.0 ± 6.4[c,e]	<0.0001
Male (%)	24.5	52.1[a]	50.0[b]	56.8[c]	<0.0001
Diabetes mellitus (%) Hypoglycemic medication use (%)	10.8 50.0	7.7 57.1	21.9[d] 55.6	12.0 69.2	0.15 0.53
Smoking status Former (%) Current (%)	 21.2 13.1	 29.4 16.8	 15.6 15.6	 31.9[c] 15.8	0.04 0.02 0.76
Alcohol use (g/day)	10.3 ± 14.9	19.8 ± 23.8[a]	11.3 ± 17.8[d]	20.2 ± 25.2[c]	<0.0001
Physical activity level Sedentary (%) Light (%)	 11.9 50.4	 7.6 45.4	 15.6 34.4	 18.8[c,e] 43.9	0.03 0.02 0.25
BMI (kg/m^2)	27.8 ± 4.1	27.6 ± 3.7	27.4 ± 4.5	27.5 ± 4.2	0.75
Height (cm)	157.2 ± 8.5	161.4 ± 9.5[a]	157.0 ± 10.4[d]	161.0 ± 9.7[c]	<0.0001
Weight (kg)	68.7 ± 11.4	71.9 ± 11.5[a]	68.1 ± 14.6	71.2 ± 12.6[c]	.03
Hypertension (%)	56.2	58.8	68.8	70.9[c,e]	0.004
SBP (mmHg)	147.2 ± 19.2	149.1 ± 20.6	150.2 ± 16.9	150.9 ± 18.2[c]	0.07
DBP (mmHg)	82.6 ± 8.1	84.2 ± 8.3	83.3 ± 8.4	85.4 ± 8.7[c]	0.002
PAD (%)	7.4	6.3	10.0	6.9	0.91
CHF (%)	0.5	3.3	0.0	5.3[c]	0.03
MI (%)	2.9	5.9	12.5[b]	3.3[f]	0.05
Stroke (%)	2.5	4.2	3.1	3.4	0.75
Thyroid disease (%)	10.7	2.5[a]	3.1	7.1	0.03
Total cholesterol (mg/dL)	226.7 ± 37.9	220.6 ± 40.5	213.4 ± 30.1[b]	216.4 ± 40.1[c]	0.04
Creatinine (mg/dL)	0.35 ± 0.48	0.34 ± 0.48	0.34 ± 0.48	0.40 ± 0.51	0.69
IL-6 (pg/mL)	2.9 ± 1.8	3.4 ± 2.3[a]	3.6 ± 2.7[b]	3.6 ± 2.4[c]	0.0005
TNF-α (pg/mL)	4.2 ± 2.1	5.5 ± 2.9[a]	4.4 ± 2.5	5.1 ± 2.9[c]	<0.0001
Folate level (nmol/L)	8.2 ± 4.8	7.8 ± 4.3	7.8 ± 5.1	6.6 ± 3.4[c,e]	<0.0001
Vitamin B6 level (nmol/L)	34.6 ± 27.7	32.5 ± 21.2	21.1 ± 11.2[b,d]	24.5 ± 13.0[c,e]	<0.0001
Vitamin B12 level (pmol/L)	397.3 ± 288.6	333.3 ± 248.2[a]	291.2 ± 157.5[b]	298.7 ± 221.2[c]	<0.0001
Hcy level (μmol/L)	10.9 ± 1.4	14.9 ± 2.2[a]	11.7 ± 1.1[b,d]	18.7 ± 6.4[c,e,f]	<0.0001
Change in Hcy (μmol/L)	-0.6 ± 1.5	-3.6 ± 2.7[a]	3.5 ± 3.3[b,d]	-0.4 ± 5.9[e,f]	<0.0001

Pairwise: $p<0.05$ for a: High to Normal vs. Stay Normal; b: Normal to High vs. Stay Normal; c: Stay High vs. Stay Normal; d: Normal to High vs. High to Normal; e: Stay High vs. High to Normal; f: Stay High vs. Normal to High
High homocysteine: ≥13 μmol/L; Normal homocysteine: <13 μmol/L

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PAD: peripheral arterial disease; CHF: congestive heart failure; MI: myocardial infarction

Table 5. Motor nerve function by vitamin B12 & homocysteine levels at baseline

	B12			Hcy		
	Normal (n=290)	Low (n=210)	p-value	Normal (n=223)	High (n=286)	p-value
CMAP (mV)	6.4 + 3.1	6.7 + 3.4	0.29	6.8 + 3.2	6.4 + 3.2	0.16
CMAP <1 mV (%)	2.4	2.9	0.76	1.8	3.2	0.34
NCV (m/s)	44.6 + 3.9	44.4.+ 3.8	0.67	44.8 + 4.2	44.1 + 3.7	0.04
NCV <40 m/s (%)	7.9	10.5	0.33	8.5	10.1	0.54

Low B12: <260 pmol/L; High homocysteine: ≥13 μmol/L

Table 6. Motor nerve function by change in B12 levels

	Change in B12				
	Stay Normal (n=245)	Low to Normal (n=63)	Normal to Low (n=50)	Stay Low (n=149)	p-value
CMAP (mV) at baseline	6.4 + 3.1	6.2 + 3.3	6.4 + 3.1	6.9 + 3.5	.43
CMAP <1 mV at baseline (%)	2.5	4.8	2.0	2.0	.66
CMAP (mV) at 3-yr follow-up	3.9 + 2.9	3.9 + 2.8	3.9 + 3.1	3.9 + 2.8	.99
CMAP <1 mV at 3-yr follow-up (%)	9.8	8.9	12.2	11.6	.90
CMAP (mV) at 6-yr follow-up	3.8 + 2.3	3.9 + 2.7	3.5 + 2.3	4.1 + 2.4	.64
CMAP <1 mV at 6-yr follow-up (%)	14.0	10.7	20.9	11.1	.37
NCV (m/s) at baseline	44.6 + 3.9	44.6 + 3.8	44.4 + 3.9	44.3 + 3.8	.90
NCV <40 m/s at baseline (%)	7.9	6.5	8.0	12.2	.44
NCV (m/s) at 3-yr follow-up	44.0 + 4.8	42.9 + 4.2	43.5 + 4.4	43.5 + 4.4	.46
NCV <40 m/s at 3-yr follow-up (%)	17.7	16.1	14.3	16.3	.95
NCV (m/s) at 6-yr follow-up	40.1 + 4.0	39.3 + 4.0	38.9 + 4.1	39.4 + 3.8	.12
NCV <40 m/s at 6-yr follow-up (%)	44.6	53.7	58.1	54.1	.19

Pairwise: p<0.05 for a: Low to Normal vs. Stay Normal; b: Normal to Low vs. Stay Normal; c: Stay Low vs. Stay Normal; d: Normal to Low vs. Low to Normal; e: Stay Low vs. Low to Normal; f: Stay Low vs. Normal to Low
Low B12: <260 pmol/L

Table 7. Motor nerve function by change in Hcy levels

	Change in Hcy				
	Stay Normal (n=205)	High to Normal (n=94)	Normal to High (n=19)	Stay High (n=196)	p-value
CMAP (mV) at baseline	6.8 + 3.2	6.4 + 3.1	6.4 + 3.8	6.4 + 3.3	0.63
CMAP <1 mV at baseline (%)	1.0	3.2	10.5[b]	3.1	0.06
CMAP (mV) at 3-yr follow-up	4.1 + 2.8	4.2 + 3.4	4.4 + 3.8	3.7 + 2.7	0.70
CMAP <1 mV at 3-yr follow-up (%)	8.2	16.5[a]	14.3	9.8	0.21
CMAP (mV) at 6-yr follow-up	3.8 + 2.4	4.1 + 2.6	3.7 + 2.8	3.8 + 2.2	0.92
CMAP <1 mV at 6-yr follow-up (%)	15.2	7.0	26.7[d]	14.1	0.12
NCV (m/s) at baseline	45.1 + 4.1	44.4 + 3.5	42.4 + 4.7[b]	44.0 + 3.8[c]	0.004
NCV <40 m/s at baseline (%)	6.4	6.5	31.6[b,d]	11.5[f]	0.002
NCV (m/s) at 3-yr follow-up	44.5 + 4.2	44.3 + 4.9	41.8 + 4.2[b]	42.6 + 4.7[c,e]	0.001
NCV <40 m/s at 3-yr follow-up (%)	11.0	12.9	28.6	23.8[c,e]	0.006
NCV (m/s) at 6-yr follow-up	40.4 + 3.8	39.6 + 3.9	36.9 + 5.3[b]	39.2 + 3.8[c,f]	0.001
NCV <40 m/s at 6-yr follow-up (%)	42.5	50.6	66.7	56.8[c]	0.03

Pairwise: p<0.05 for a: High to Normal vs. Stay Normal; b: Normal to High vs. Stay Normal; c: Stay High vs. Stay Normal; d: Normal to High vs. High to Normal; e: Stay High vs. High to Normal; f: Stay High vs. Normal to High
High homocysteine: ≥13 μmol/L

Table 8. Multivariable mixed models– CMAP (mV) longitudinal trend and change in Hcy

Change in Hcy	**β**	**p-value**
High to Normal	-.16	.46
Normal to High	-.22	.62
Stay High	-.41	**.04**

Ref=Stay Normal
Adjusted for age, sex, site, diabetes, and folate level

3.0 THE RELATIONSHIP OF VITAMIN B12 AND PERIPHERAL SENSORY AND MOTOR NERVE FUNCTION: IS THERE A THRESHOLD EFFECT?

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3.1 ABSTRACT

Objective: To examine whether poor vitamin B12 levels (i.e., low or deficient) are associated with worse peripheral sensory and motor nerve function in older adults and whether a threshold serum B12 level exists for optimal nerve function.

Methods: Participants were from the Health, Aging, and Body Composition Study. We included 2287 participants [mean age: 76.5 ± 2.9 years; 51.4% female; 38.3% black] from the 2000-2001 visit with B12 levels and ≥1 peripheral nerve measure: 1.4g/10g monofilament detection; average vibration threshold; peroneal nerve conduction amplitude and velocity [NCV]; and symptoms (numbness; aching/burning pain). Low B12 was determined if <260 pmol/L and deficient if B12 <260 pmol/L & methylmalonic acid [MMA] >271 nmol/L & MMA >2-methylcitrate].

Results: Low B12 prevalence was 17.1%, and 7.0% were deficient. Low B12 was associated with greater insensitivity to light (1.4g) touch (OR=1.28; 95% CI=[1.01,1.62]), after adjusting for age, sex, race, diabetes, height, alcohol use, and 3MS score, and with worse NCV [42.8m/s vs. 43.5m/s] (β =-0.63; p=.04), after adjusting for covariates above plus weight, ankle arm index, and systolic blood pressure. Associations were consistent for deficient B12. A threshold B12 level of 390 pmol/L for NCV was significant after adjustment.

Conclusions: Poor B12 is associated with worse sensory and motor peripheral nerve function. Serum B12 levels, higher than current clinical cutpoints, may be necessary for optimal

peripheral nerve function. Worse nerve function may lead to impaired physical function and disability in older adults. Supplemental B12 is efficacious in older adults, and may correct B12 deficits associated with peripheral nerve impairments.

3.2 INTRODUCTION

Vitamin B12 deficiency affects 5-20% of older adults and low B12 levels are highly prevalent among older adults, affecting up to 40% (Baik 1999; Varela-Morieras 2009; Andres 2004; Park 2006). Studies have reported a wide range of prevalence rates, due to different populations studied, but also because of a lack of agreement on diagnostic criteria for low or deficient B12 levels. Studies use different cutpoints for low or deficient B12 level (e.g., 148 pmol/L for deficiency [also used as the clinical cutpoint] and 260 pmol/L for low B12) and some use methylmalonic acid to determine B12 deficiency. Importantly, the cutpoint used for low B12 was not selected based on physiological or pathological endpoints that could be interpreted as a consequence of B12 deficiency.

More than half of older adults with B12 deficiency have food-cobalamin malabsorption (i.e., impaired digestion and absorption of protein-bound B12) (Andres 2004; Andres 2005; Allen 2009). In the 2010 Dietary Guidelines for Americans, the Recommended Daily Allowance of vitamin B12 for older adults is 2.4 μg, the majority from fortified foods and supplements (USDA & USDHHS, 2010). Other causes of low or deficient B12 levels are pernicious anemia, insufficient intake, gastric surgery, gastrointestinal disease, and certain medications (e.g., proton-pump inhibitors, metformin) (Andres 2004).

B12 deficiency has been associated with hematological disorders (e.g., megaloblastic anemia) and neuropsychological disorders (e.g., dementia, cognitive impairment, depression) (Andres 2004; Bernard 1998; Baik 1999; Johnson 2003). B12 deficiency can cause demyelination (Weir 1999) and has been associated with peripheral neuropathy, loss of sensation in peripheral nerves, and weakness in lower extremities in older adults (Baik 1999; Klee 2000; Saperstein 2002; Volkov 2006; Mold 2004). In particular, vitamin B12 deficiency is associated with large fiber (type A) neuropathy; type A nerve fibers act as both sensory and motor fibers (Kumar 2004). When assessing sensory peripheral nerve function and symptoms, there is a level of participation, thus poor cognitive function may be a potential confounder.

In older adults, there is a high prevalence of poor peripheral nerve function and neuropathy and the prevalence increases with age (Baldereschi 2007; Rivner 2001; Resnick 2001; Gregg 2004). Worse peripheral nerve function is related to lower strength, lower bone mineral density, and lower physical performance in older adults, which may potentially lead to disability in older adults (Strotmeyer 2008; Strotmeyer 2009; Inzitari 2006; Resnick 2000). Thus, identifying risk factors for poor peripheral nerve function is crucial. While B12 deficiency is a recognized risk factor for clinical peripheral neuropathy, little is known about the relationship between low B12 levels and subclinical peripheral sensory and motor nerve function in older adults. Furthermore, a critical level of serum B12 has not been determined for optimal peripheral nerve function. The purpose of this study is 1) to examine whether low or deficient vitamin B12 levels are associated with worse peripheral sensory and motor nerve function in older adults and 2) to determine whether there is a threshold effect of vitamin B12 levels on peripheral sensory and motor nerve function.

3.3 METHODS

3.3.1 Study population

We conducted a cross-sectional study of B12 and peripheral nerve function in 2287 participants of the Health, Aging and Body Composition (Health ABC) Study. The Health ABC Study is an ongoing, prospective cohort study of 3,075 well-functioning black and white men and women, aged 70-79 years at the 1997-1998 baseline examination, studying associations between change in body composition and disease. Participants were recruited from a random sample of Medicare-eligible white adults and all eligible black community-dwelling residents in Pittsburgh, PA, and Memphis, TN. Individuals were ineligible if they had difficulty walking a ¼ mile (400m), climbing 10 steps, performing activities of daily living; had life-threatening cancer or treatment for cancer in the last 3 years; or were planning to move out of the study area within 3 years. The study was approved by the Institutional Review Boards at the University of Pittsburgh and the University of Tennessee Health Science Center, and informed consent was obtained from all participants.

Of 3075 participants at baseline, 2405 had a 2000-2001 clinic examination. The remaining cohort had a home visit (n=88), telephone follow-up (n=233), proxy interview only (n=49), were deceased (n=187), withdrew (n=9), or missed the examination (n=104). We excluded participants missing all peripheral nerve function measures (n=1), vitamin B12 levels (n=98), fasting blood glucose levels (n=14) or with diabetes onset at ≤20 years of age (n=5). Thus, 2287 participants (48.6% male, 38.3% black) had serum B12 measured and at least one peripheral nerve measure, representing 74.4% of baseline participants and 95.1% of those attending the 2000-2001 examination.

3.3.2 Assays

Blood samples were frozen at -70˚C in cryogenic vials. Analyses for vitamin B12 (using 300 ul sera from the 2000-01 visit) were performed at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, University of Vermont, by a competitive immunoassay on the ADVIA Centaur (Bayer HealthCare, LLC) using direct chemiluminescent technology. The normal range was 72 to 1427 pmol/L determined from 272 serum samples. The assay CV ranged from 4% to 10% and a 6.7% CV was observed for the 5% of the sample blinded for quality control.

If serum B12 was <260 pmol/L, serum methylmalonic acid (MMA), total homocysteine (Hcy), serum 2-methylcitrate (2-MCA), and cystathionine were tested. Vials from the same blood draw for vitamin B12 were shipped to the University of Colorado Health Sciences Center for the metabolite assays and were analyzed by capillary gas chromatography-mass spectrometry (Stabler 1999). The normal ranges were 73-271 nmol/L for MMA, 5.4-13.9 µmol/L for Hcy, 60-228 nmol/L for 2-MCA, and 44-342 nmol/L for cystathionine. Inflammatory markers, TNF-α and IL-6, were measured at the 1997-1998 and 2000-2001 visit, respectively (Cesari 2003).

Categories for vitamin B12 status used in the analyses included: 1) Deficient: B12 <260 pmol/L and MMA >271 nmol/L and MMA >2-methylcitrate; Low: B12 <260 pmol/L & (MMA ≤271 nM or MMA ≤2-MCA); Normal B12: ≥260 pmol/L; (Stabler 1986); and 2) Low: B12 <260 pmol/L; Normal B12: ≥260 pmol/L (Tucker 2005).

3.3.3 Peripheral nerve function

Sensory and motor peripheral nerve function was measured on the right leg/foot unless contraindicated by amputation, knee replacement, surgery, trauma, or ulcer. Monofilament

testing was done on the dorsum of the great toe. Reduced sensation was determined if participants were unable to detect 3 out of 4 touches for each 1.4g (light) and 10g (standard) monofilaments. Average vibration threshold in micrometers was recorded at the great toe (VSA-3000 vibratory sensory analyzer; Medoc). Nerve conduction amplitude in millivolts (compound motor action potential [CMAP]) and velocity in meters per second (nerve conduction velocity [NCV]) were measured between the popliteal fossa and ankle (NeuroMax 8; XLTEK). To assess symptoms of peripheral neuropathy (PN), participants were asked "in the past 12 months, have you ever had: 1) numbness, an asleep feeling, or a prickly feeling in your legs or feet? (yes/no); 2) sudden stabbing, burning pain or a deep aching in your legs or feet? (yes/no)."

3.3.4 Potential confounders

Potential confounders were identified if they were associated with vitamin B12 and peripheral nerve function and were included as covariates in the multivariable analyses. Demographic and health information including alcohol use, cerebrovascular disease, coronary heart disease, congestive heart failure, and peripheral arterial disease (determined by self-reported physician diagnosis) were assessed at the 1997-1998 visit, as well as processing speed, using the Digit Symbol Substitution test (DSST). Thyroid stimulating hormone was measured at the 1998-1999 visit. Global cognitive function, measured with the Modified Mini-Mental State Examination (3MS); smoking status; medications (e.g., fibrate, niacin, statin, thyroid, metformin); and B12 supplement use (e.g., multivitamin or supplemental B12 [oral or intramuscular]) were assessed during the 1999-2000 visit. Age, diabetes mellitus (determined by fasting glucose, medications, self-reported physician diagnosis), hypertension (determined by physiological exam, medications, and self-reported physician diagnosis), blood pressure, cholesterol, ankle-arm index

(low: <0.9; normal: 0.9-<1.3; high: ≥1.3), height, weight, fat mass, lean mass, physical activity from walking and stair-climbing (kcal/kg/wk), high cystatin-C (≥1 mg/L) were assessed during the 2000-2001 year visit. Body mass index (BMI) was calculated as weight (kg)/ height^2 (m^2). Whole body bone mineral–free lean mass and fat mass were assessed by DXA (Hologic 4500A, software version 9.03; Hologic).

3.3.5 Statistical analyses

Differences in demographics, lifestyle factors, chronic health conditions, cardiovascular risk factors, medication and supplement use, and body composition were tested by vitamin B12 status, using Pearson χ^2 tests or Fisher's exact test for categorical variables and Kruskal-Wallis, ANOVA, or t-tests for continuous variables.

Logistic regression was performed for ability to detect 1.4g (light) and 10g (standard) monofilaments, and PN symptoms. Linear regression modeling was done for nerve conduction amplitude and velocity. Tobit regression was used for average vibration threshold because of a ceiling effect.

Regression models were performed separately for each peripheral nerve function variable with vitamin B12 status as the predictor variable. The models were built progressively in order, adjusting for potential confounders: demographics, diabetes, lifestyle factors, body composition, physiological factors, medication and supplement use, chronic health conditions, and inflammatory markers. DSST and 3MS were additionally included for vibration threshold, monofilament detection, and PN symptoms, due to cognitive aspects of these measures. Age, gender, race, clinic site, and diabetes were adjusted for in all models; other variables were removed if $p > 0.10$. Sensitivity analyses was conducted excluding participants with diabetes,

and we performed analyses using two additional definitions for vitamin B12 in order to confirm whether results were consistent for other definitions used for B12 deficiency: 3) Deficient: B12 <148 pmol/L (Tucker 2005); 4) Deficient: B12 <148 pmol/L or [normal renal function and Hcy ≥13.9 μmol/L], estimating normal renal function using MMA <271 nmol/L and [Hcy ≥13.9 μmol/L or (cystathionine ≤342 nmol/L and 2-MCA ≤228 nmol/L)]. Results were not significant; however, we had a very small number of participants with B12 deficiency using these definitions (26 participants [1.1%] using definition 3, and 55 participants [2.4%] using definition 4. Multicollinearity for independent variables was assessed using the variance inflation factor (VIF); no VIF was > 2.

Threshold analysis was performed using restricted cubic spline linear regression with 5 knots to determine whether there is a threshold serum vitamin B12 level for peripheral nerve function. The knots were placed at the 5^{th}, 25^{th}, 50^{th}, 75^{th} and 95^{th} percentiles of serum B12 levels. To determine whether the threshold levels were significant, slopes before and after the cutpoint were compared using fully adjusted regression models. All analyses were conducted with SAS, version 9.2 (SAS Institute Inc, Cary, NC), using adapted code for the threshold analysis (Desquilbet 2010).

3.4 RESULTS

The prevalence of low vitamin B12 levels (<260 pmol/L) was 17.1%, and B12 deficiency, using definition 1 with MMA, was found in 7.0% of the participants. Thus, 10.1% had low but not deficient B12 levels. Table 9 shows the descriptive characteristics by the 3-level variable for B12 deficiency (i.e., deficient B12, low but not deficient, and normal B12). Participants with

B12 deficiency were more likely to be older, male, white, have lower cholesterol levels, and were less likely to take supplemental B-12, compared to those with normal B12 levels. Those with B12 deficiency were more likely to be white and have lower serum B12 levels compared to those with low but not deficient B12 levels. Among the participants taking a supplement containing vitamin B12, 96% were taking a multivitamin. About 8% of those with normal B12 levels were specifically taking a B12 supplement (oral or intramuscular) and 3% of those with low or deficient B12.

Nearly half (45.6%) of the participants were unable to detect 1.4g monofilament; 5.9% were unable to feel the maximum vibration (130 μ); 11.0% had poor CMAP (<1 mV); and 22.2% had poor NCV (<40 m/s). Table 10 demonstrates peripheral nerve function by vitamin B12 status. Participants with vitamin B12 deficiency were less likely to detect 1.4g monofilament, and more likely to have worse (higher) average vibration threshold detection and lower nerve conduction velocity compared to those with normal B12 levels. There was no difference in nerve function between those with B12 deficiency and those with only low B12 levels. There were no differences between poor (i.e., low or deficient) and normal B12 and ability to detect standard (10g) monofilament, CMAP amplitude, or peripheral neuropathy symptoms.

Tables 11-14 illustrates the multivariable regression results using the 3-level definition for deficient B12 and 2-level definition for low B12. Associations were consistent for B12 deficiency (using MMA) and low B12 (using serum B12 only). Inability to detect light (1.4g) monofilament was associated with deficient or low B12, after adjusting for age, sex, race, site, DM, height, alcohol use, and 3MS score [Table 11]. Deficient and low B12 was associated univariately with worse vibration detection, but was not associated with B12 after adjusting for

potential confounders. Sex, race, site, DM, and 3MS score attenuated the association [Table 12]. Table 13 shows that CMAP was univariately associated with low B12, but after adjustment, the relationship was no longer significant. Worse NCV was associated with deficient and low B12 levels, after adjusting for age, sex, race, site, DM, height, weight, alcohol use, AAI, and SBP [Table 14]. The category of "low but not deficient B12" (B12 <260 pM & (MMA≤271 nM or MMA ≤2-MCA), was not statistically associated with nerve function for any outcome. There were no significant associations found between B12 status and standard (10g) monofilament detection or PN symptoms (data not shown). Sensitivity analysis was performed removing participants with diabetes, and the results were consistent.

In the multivariable regression models, a higher 3MS score was associated with lower (better) vibration detection and being more likely to detect 1.4g monofilament. However, the correlation between 3MS or DSST and either of the sensory nerve function measures or symptoms was weak ($-0.13 < \rho < 0.10$).

Using restricted cubic spline analyses, threshold levels were evaluated for light monofilament detection, average vibration detection, CMAP, and NCV. Figure 2 shows the threshold level found for light monofilament detection was a serum B12 level of 400 pmol/L, this level was significant before adjustment (p=.04), but was not significant after fully adjusting for potential confounders (p=.07). Figure 3 demonstrates the threshold level of B12 for average vibration detection. The threshold level for vibration was 290 pmol/L which was borderline significant in the adjusted model (p=.05). Figure 4 illustrates the threshold effect of B12 on CMAP, which was found to be around 380 pmol/L, but the level was not significant. Finally, Figure 5 shows a threshold level of 390 pmol/L for NCV, which was significant after adjustment, comparing slopes before and after using a fully adjusted regression model (p=.02).

3.5 DISCUSSION

In our analyses, we found that older adults with poor B12 had both worse sensory and motor peripheral nerve function. A threshold serum B12 level of 390 pmol/L for NCV was significant after adjustment, suggesting higher serum B12 levels, above current guidelines, may be needed for optimal nerve function in older adults. Our results suggest that using MMA with serum B12 may be best, since we found stronger associations with deficient B12, rather than using serum B12 only (<260 pmol/L) and low but not deficient B12 levels was not significantly associated with any nerve measure. However, our findings also suggest that using serum B12 only for <260 pmol/L is sufficient, since the associations were consistent with using serum B12 with MMA. This study is unique in comparing different definitions of vitamin B12 status and their relationship with motor and sensory nerve function and examining whether there is a threshold serum B12 level that most effectively identified persons with higher peripheral nerve function. This work is vital because a threshold effect of vitamin B12 on nerve function has not been tested. Worse nerve function may lead to impaired physical function and disability in older adults (Resnick 2000; Resnick 2001; Strotmeyer 2008).

While only a threshold B12 level of 390 pmol/L for NCV was found to be significant after adjustment for potential confounders, very similar threshold levels were found for light monofilament detection (400 pmol/L) and CMAP amplitude (380 pmol/L). These threshold levels were higher than expected and even 290 pmol/L, the threshold level found for vibration detection, is higher than the commonly used "low" cutpoint (260 pmol/L). This suggests that older adults may need to maintain higher B12 levels for optimal peripheral nerve function.

The prevalence of poor NCV (22.2%) was more than twice as high as poor CMAP (11.0%). Low CMAP is related to nerve axonal damage and low NCV is related to nerve

demyelination. We expected the prevalence of poor NCV to be higher than poor CMAP since demyelination is thought to occur before axonal degeneration because the myelin surrounds the axon and provides protection (Arezzo 2004).

There was no association with vitamin B12 status and ability to detect standard (10g) monofilament or peripheral neuropathy symptoms. Light monofilament may be more sensitive compared to using standard (10g) monofilament, detecting sensory neuropathy at an earlier stage (Bourcier 2006; Thomson 2008). The symptoms of numbness and deep aching/burning pain in the legs or feet may not be specific for peripheral neuropathy in older adults in the context of B12 deficits, and peripheral neuropathy is sometimes asymptomatic (Franse 2000).

There is still much controversy over how to define poor vitamin B12 levels. Some studies use serum B12 levels only, with cutpoints ranging from 74 to 140 pmol/L for deficiency and from 185 to 260 pmol/L for low B12; some use low serum B12 and high MMA (e.g., > 2 or 3 SD above the mean); and a few use high MMA alone (Park 2006; Carmel 2003; Carmel 1988; Baik 1999; Snow 1999; Clarke 2007). While there is still no agreed-upon cutpoint for low or deficient B12 levels, the most commonly used serum B12 cutpoints are 148 pmol/L for deficiency and 260 pmol/L for low B12. The cutpoint of 260 pmol/L was not based on an outcome associated with B12 deficiency, but instead was suggested based on a high prevalence (i.e., 27.9%) of older adults from the Framingham study with elevated MMA (i.e., > 3 SD above mean) among those whose serum B12 levels were below 260 pmol/L (Lindenbaum 1994), and this cutpoint has since been adopted by other studies (Park 2006; Tucker 2005; Carmel 2003). Low vitamin B12 levels result in elevated MMA levels, and MMA is considered to be a highly sensitive marker of B12 deficiency, though is not often used in clinical practice and it is

expensive (Stabler 1998; Savage 1994). Thus it is important to see if using only serum B12 levels is adequate.

Strengths of this analysis were that we had a large cohort of older men and women, and had MMA levels for those who had serum B12 levels <260 pmol/L (Stabler 1998). We had measures of peripheral motor and sensory nerve function. Sensory nerve function was assessed using both average vibration threshold, and also monofilament detection, which even though it is less sensitive, it is highly specific and has clinical significance (i.e., it is a low-cost and quick test that can be done in an exam room) (Feng 2009). Measuring motor nerve function with nerve conduction is considered the gold standard, because it is highly sensitive, reliable and reproducible (England 2005). Finally, we examined threshold levels of serum B12 for sensory and motor peripheral nerve function.

A limitation of this study was we had a small percentage of participants (1.1%) with deficient B12 levels, using a cutpoint of 148 pmol/L for serum B12. The participants in this study were likely healthier than older adults in the general population and 39.2% of participants took a supplement with vitamin B12. Thus we may not have had sufficient statistical power to examine a relationship between clinically deficient serum B12 (<148 pmol/L) and peripheral nerve function. We also did not have objective sensory nerve function from the nerve conduction test.

In the aftermath of the 1998 mandatory folic acid fortification in the United States, it is important to study vitamin B12 levels and consequences of poor B12 status in older adults (Varela-Moreiras 2009). Importantly, a high intake of folic acid may correct megaloblastic anemia, which is caused by a deficiency in B12 and/or folic acid (Dickinson 1995; Klee 2000). Thus it is critical to monitor vitamin B12 levels among older adults because the classic sign of

anemia may not be present. If vitamin B12 deficiency goes unnoticed, neurological damage may progress and is not easily reversible (Varela-Moreiras 2009).

We found that poor B12 levels are associated with worse sensory and motor nerve function in older adults. Higher levels of serum B12 levels (~390 pmol/L) may be needed to maintain optimal peripheral nerve function. Randomized clinical trials are needed to establish that sufficient B12 supplementation can result in better peripheral nerve function. These findings have important implications for motor functioning and disability in older adults. Several studies have shown an association between peripheral neuropathy or poor peripheral nerve function and impaired mobility and falls (Strotmeyer 2008; Resnick 2000; Richardson 1995; Richardson 1992; Koski 1998; Luukinen 1995; Sorock 1992). Supplemental B12 is easily available, adequately absorbed, highly tolerated in older adults (Andres 2010), and may potentially correct vitamin B12 deficits associated with impaired peripheral nerve function.

3.6 TABLES AND FIGURES

Table 9. Descriptive characteristics by vitamin B12 status

	Normal (n=1896)	Low only (n=232)	Deficient (n=159)	p-value
Demographics				
Age (years)	76.5 ± 2.9	76.4 ± 2.8	77.2 ± 2.9[b]	.009
Male (%)	47.0	55.6[a]	57.2[b]	.004
Black (%)	39.6	36.6	25.2[b,c]	.001
Diabetes status				.66
Impaired fasting glucose (%)	16.0	18.1	16.4	.71
Diabetes (%)	21.5	19.4	25.2	.39
Metformin use (%)	15.6	27.9[a]	23.1	.08
Lifestyle Factors				
Smoking status				.56
Former (%)	47.1	47.4	53.9	.27
Current (%)	7.0	7.9	6.5	.85
Alcohol use				.70
Former (%)	21.1	21.2	20.3	.97
<1 per week (%)	21.3	21.2	19.6	.89
1-7 per week (%)	22.7	22.1	24.1	.90
>1 per day (%)	6.7	10.2	9.5	.09
Physical activity (kcal/kg/wk)	31.4 ± 50.7	41.4 ± 76.4	26.1 ± 39.3	.11
Body Composition				
BMI (kg/m^2)	27.2 ± 4.8	27.5 ± 4.4	26.9 ± 4.2	.35
Height (mm)	1654.1 ± 93.2	1670.9 ± 99.1	1663.5 ± 94.2	.02
Weight (kg)	74.9 ± 15.1	77.9 ± 15.2	75.2 ± 14.9	.02
Total fat mass (kg)	26.1± 8.8	27.1 ± 8.4	25.6 ± 8.1	.11
Total lean mass (kg)	48.8 ± 10.2	50.5 ± 10.6	49.7 ± 10.4	.06
Physiological Factors				
SBP (mmHg)	134.9 ± 19.8	137.1 ± 20.6	135.4 ± 22.7	.40
DBP (mmHg)	71.4 ± 10.7	72.7 ± 11.2	70.9 ± 12.4	.16
Ankle-arm index				.27
Low (%)	15.8	16.4	18.1	.75
Stiffening (%)	5.6	2.7	3.2	.09
Total cholesterol (mg/dL)	192.6 ± 37.9	190.5 ± 35.7	182.1 ± 34.9	.009
Cystatin-C ≥1 mg/L (%)	44.7	46.1	56.1[b]	.02
B12 supplement use (%)	42.9	19.6[a]	22.1[b]	<.0001
Medication use				
Fibrate use (%)	1.1	0.5	1.4	.68
Niacin use (%)	1.1	0.5	0.7	.90
Statin use (%)	20.1	18.4	19.1	.81
Thyroid medication (%)	12.7	11.0	12.1	.76
History of comorbidities				
Hypertension (%)	72.7	75.1	74.7	.67
CBVD (%)	6.5	8.7	7.7	.41
CHD (%)	15.2	20.0	20.0	.07
CHF (%)	0.9	0.0	0.6	.38
PAD (%)	4.3	3.1	5.7	.45
Inflammatory markers				
IL-6 (pg/mL)	3.6 ± 3.7	3.7 ± 3.8	3.7 ± 4.4	.85
TNF-α (pg/mL)	3.4 ± 1.5	3.4 ± 1.9	3.7 ± 1.5[b]	.01

Table 9 continued

Cognitive tests				
3MS score	90.4 ± 8.1	90.8 ± 7.2	90.0 ± 7.7	.50
DSST score	36.8 ± 14.8	35.8 ± 13.9	35.2 ± 11.9	.15
B12 level (pmol/L)	474.5 ± 203.0	223.2 ± 30.4[a]	200.7 ± 39.8[b,c]	<0.0001

Pairwise: p<0.05 for a: Low vs. Normal; b: Deficient vs. Normal; c: Deficient vs. Low; deficient B12: B12<260 pmol/L & MMA>271nmol/L and MMA>2-methylcitrate

Low only: B12 <260 pM & (MMA ≤271 nM or MMA ≤2-MCA) Deficient: B12 <260 pM & MMA >271 nM & MMA >2-MCA

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; CBVD: cerebrovascular disease; CHD: coronary heart disease; CHF: congestive heart failure; PAD: peripheral arterial disease; 3MS: modified Mini-Mental Examination; DSST: Digit Symbol Substitution Test

Table 10. Peripheral sensory and motor nerve function by vitamin B12 status

	Normal (n=1896)	Low only (n=232)	Deficient (n=159)	p-value
Monofilament				
Unable to detect 10g (%)	8.8	8.3	9.0	.97
Unable to detect 1.4g (%)	44.4	48.3	56.1[b]	.01
Vibration threshold (μ)	50.5 ± 35.3	53.8 ± 36.9	59.8 ± 36.4[b]	.003
Unable to detect vibration (%)	5.7	5.8	7.9	.54
CMAP (mV)	3.4 ± 2.0	3.2 ± 1.9	3.0 ± 2.0	.14
<1 mV (%)	10.7	12.0	13.7	.54
NCV (m/s)	43.8 ± 5.4	43.1 ± 5.5	42.2 ± 5.2[b]	.006
<40 m/s (%)	21.6	23.0	28.7	.21
Numbness (%)	29.3	28.1	25.2	.53
Aching/burning pain (%)	16.8	19.8	13.8	.29

Pairwise: p<0.05 for a: Low vs. Normal; b: Deficient vs. Normal; c: Deficient vs. Low; deficient B12: B12<260 pmol/L & MMA>271nmol/L and MMA>2-methylcitrate

Low only: B12 <260 pM & (MMA ≤271 nM or MMA ≤2-MCA) Deficient: B12 <260 pM & MMA >271 nM & MMA >2-MCA

Table 11. Multivariable logistic regression: Inability to detect 1.4g monofilament

	Low only: B12 <260 pM & (MMA ≤271 nM or MMA ≤2-MCA) Deficient: B12 <260 pM & MMA >271 nM & MMA >2-MCA				Low: B12 <260 pmol/L	
	Low only		**Deficient**		**Low**	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Model 1: unadjusted	1.17	[0.89, 1.54]	1.60	**[1.15, 2.23]**	1.33	**[1.07, 1.65]**
Model 2: adj. for demographics	1.13	[0.86, 1.50]	1.51	**[1.08, 2.11]**	1.27	**[1.01, 1.59]**
Model 3: fully adjusted*	1.15	[0.85, 1.54]	1.50	**[1.06, 2.13]**	1.28	**[1.01, 1.62]**

*Adjusted for demographics (age, sex, race, site), DM, height, alcohol use, 3MS score

Table 12. Multivariable linear regression: Mean vibration detection (μ)

	Low only: B12 <260 pM & (MMA ≤271 nM or MMA ≤2-MCA) Deficient: B12 <260 pM & MMA >271 nM & MMA >2-MCA				Low: B12 <260 pmol/L (Definition 2)	
	Low only		**Deficient**		**Low**	
	β	**p**	**β**	**p**	**β**	**p**
Model 1: unadjusted	3.37	.21	9.68	**.002**	5.91	**.006**
Model 2: adj. for demographics	1.97	.43	5.23	.08	3.28	.11
Model 3: fully adjusted*	1.27	.61	5.23	.08	2.88	.15

Ref: normal

*Adjusted for demographics (age, sex, race, site), DM, height, weight, AAI, high cystatin-C, 3MS score, vibration variance

Table 13. Multivariable linear regression: Mean CMAP (mV)

	Low only: B12 <260 pM & (MMA ≤271 nM or MMA ≤2-MCA) Deficient: B12 <260 pM & MMA >271 nM & MMA >2-MCA				Low: B12 <260 pmol/L (Definition 2)	
	Low only		**Deficient**		**Low**	
	β	**p**	**β**	**p**	**β**	**p**
Model 1: unadjusted	-.176	.26	-.347	.06	-.247	**.04**
Model 2: adj. for demographics	-.120	.44	-.187	.30	-.148	.23
Model 3: fully adjusted*	-.127	.40	-.159	.38	-.140	.25

Ref: normal

*Adjusted for demographics (age, sex, race, site), DM, height, AAI, cholesterol level, high cystatin-C

Table 14. Multivariable linear regression: Mean NCV (m/s)

	Low only: B12 <260 pM & (MMA ≤271 nM or MMA ≤2-MCA) Deficient: B12 <260 pM & MMA >271 nM & MMA >2-MCA				Low: B12 <260 pmol/L (Definition 2)	
	Low only		**Deficient**		**Low**	
	β	**p**	**β**	**p**	**β**	**p**
Model 1: unadjusted	-.670	.13	-1.58	**.002**	-1.04	**.003**
Model 2: adj. for demographics	-.280	.48	-.891	.06	-.530	.09
Model 3: fully adjusted*	-.256	.52	-1.16	**.01**	-.626	**.04**

Ref: normal

*Adjusted for demographics (age, sex, race, site), DM, height, weight, alcohol use, AAI, SBP

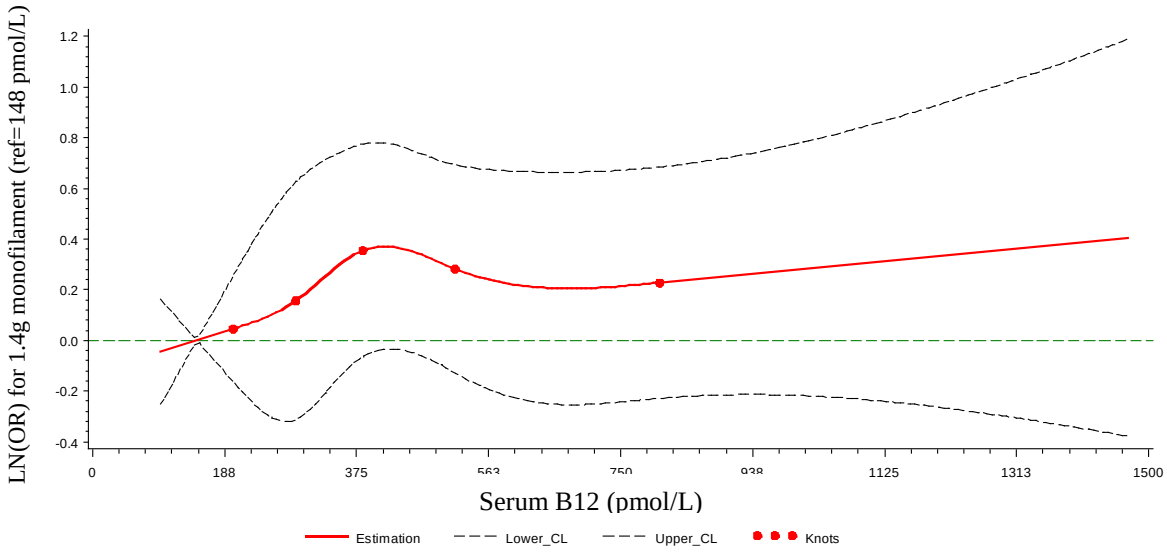

Figure 2. Threshold of serum B12 level for light (1.4g) monofilament

*Adjusted for age, sex, race, site, DM, height, alcohol use, 3MS score

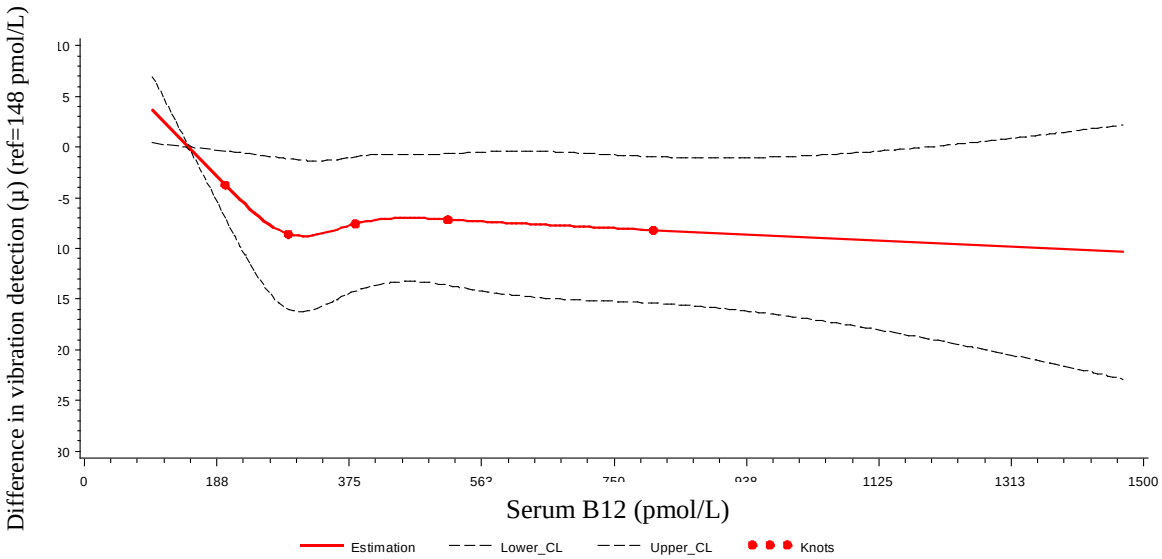

Figure 3. Threshold of serum B12 level for average vibration detection

*Adjusted for age, sex, race, site, DM, height, weight, AAI, high cystatin-C, 3MS score, vibration variance

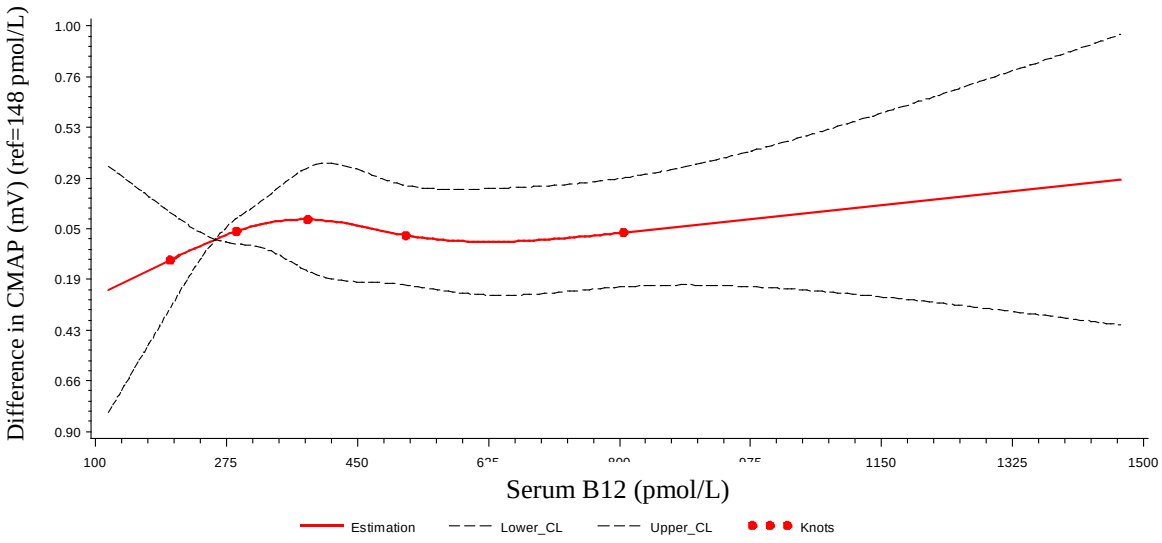

Figure 4. Threshold of serum B12 level for CMAP

*Adjusted for age, sex, race, site, DM, height, AAI, cholesterol level, high cystatin-C

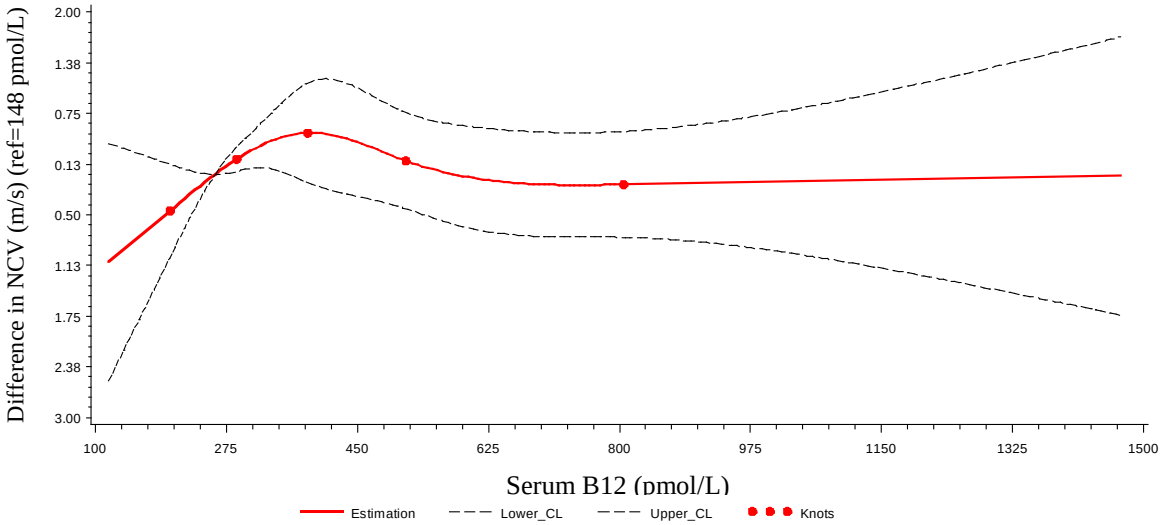

Figure 5. Threshold of serum B12 level for NCV

*Adjusted for age, sex, race, site, DM, height, weight, alcohol use, AAI, SBP

4.0 LOW VITAMIN B12 LEVELS AND 6-YEAR DECLINE IN INFORMATION PROCESSING SPEED IN OLDER ADULTS

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4.1 ABSTRACT

Objectives: Vitamin B12 deficiency may cause demyelination, resulting in white matter damage and slower information processing speed. The purpose of this analysis is to examine whether low initial vitamin B12 levels [<260 pmol/L] or 7-year change in vitamin B12 levels are associated with a greater decline in processing speed, and to determine whether there is a threshold effect of serum B12 on processing speed decline.

Methods: 2123 participants from the Health, Aging, and Body Composition Study (mean age: 76.5±2.9 years; 48.2% male; 36.8% black) had serum B12 levels at the 2000-01 visit and Digit Symbol Substitution Test [DSST] scores at the 2001-02 visit. Of these, 1033 participants (mean age: 76.0±2.8 years; 47.9% male; 31.4% black) also had serum B12 levels and DSST scores at the 2007-08 visit and were included in the longitudinal analysis.

Results: Low initial B12 (n=173; 16.7%) was associated with greater annualized DSST decline, adjusting for initial DSST scores, demographics, socioeconomic and lifestyle factors, CES-D score, medication, and chronic health conditions (β=-0.267; p=.04). Improving to normal B12 (n=82) was associated with greater annualized DSST decline compared to sustained normal B12 (n=792), after adjustment (β=-0.465; p=.01). Similar associations were found for decline defined as >1SD from mean change. A threshold level of 410 pmol/L for serum B12 was found to be associated with lower DSST decline (p=.01).

Conclusions: Initial low B12 <260 pmol/L was associated with greater DSST score declines in older adults. Preventing initial low B12 levels is critical and higher levels (~410 pmol/L) may be optimal. These results are important since cognitive impairments associated with prolonged low B12 levels potentially may not be reversible.

4.2 INTRODUCTION

The prevalence of vitamin B12 deficiency is 5-20% in older adults, and low B12 levels are found in up to 40% of older adults (Baik 1999). Many studies have examined the relationship between vitamin B12 and cognition; however, the results have been inconsistent (Kang 2006). Some studies have found an association between vitamin B12 and cognition (Elias 2006; Johnson 2003; Goodwin 1983; Wahlin 2001; Riggs 1996; Hin 2006), while others have not (Kang 2006; Teunissen 2003). Most of the longitudinal studies on the relationship between B12 and cognitive function in older adults have found no association (Kado 2005; Mooijaart 2005; Kang 2006); however, in a longitudinal study of older adults, low vitamin B12 status (e.g., low holotranscobalamin, high homocysteine, and high methylmalonic acid), but not serum B12, was associated with lower mini-Mental State Examination [MMSE] scores at both baseline and after 10 years (Clarke 2007). In a randomized clinical trial of older adults with mild cognitive impairment, B vitamins (i.e, folic acid, B6, B12) slowed the rate of brain atrophy (Smith 2010), and in another, B12 supplementation improved cognitive and cerebral function in older adults with B12 deficiency (van Asselt 2001). However, many randomized clinical trials found B vitamins do not improve cognitive performance (McMahon 2006; Stott 2005; Eussen 2006), or slow the rate of cognitive decline (Aisen 2008).

The physiologic mechanism by which low B12 may negatively affect cognition is not fully understood (Malouf 2009). B12 is essential to convert homocysteine to methionine which is then converted to S-adenosylmethionine [SAM] (de Lau 2009). Low B12 may cause a deficiency in SAM, which results in oxidative damage, neurotransmitters dysregulation (e.g., dopamine, serotonin, melatonin), and impaired cognition. Elevated homocysteine levels may be toxic to neurons and cause vascular damage (Malouf 2009). Finally, B12 deficiency can damage

myelin as a result of deficient methylation of myelin basic protein (Weir 1999). Demyelination may cause white matter damage and slow processing speed (Smith 2009).

Interestingly, most studies on the relationship between B12 status and cognitive impairment did not use cognitive tests that assess processing speed, the brain function most likely to be affected by vascular and white matter damage. The Digit Symbol Substitution Test [DSST] assesses information processing speed and psychomotor performance (Wechsler 1981). The aims of this study are 1) to examine whether low vitamin B12 levels, rather than deficient levels, are associated with slower processing speed in older adults; 2) to determine if low initial vitamin B12 levels or 7-year change in vitamin B12 levels are associated with decline in processing speed; and 3) to detect whether there is a threshold effect of serum B12 on decline in processing speed.

4.3 METHODS

4.3.1 Study population

Participants were from the Health, Aging, and Body Composition [Health ABC] Study. The Health ABC Study is an ongoing, prospective cohort study of 3,075 well-functioning black and white men and women, aged 70-79 years at the 1997-1998 baseline examination, studying associations between change in body composition and disease. Participants were recruited from a random sample of Medicare-eligible white adults and all eligible black community-dwelling residents in Pittsburgh, PA, and Memphis, TN. Individuals were ineligible if they had difficulty walking a ¼ mile (400m), climbing 10 steps, performing activities of daily living; had life-

threatening cancer or treatment for cancer in the last 3 years; or were planning to move out of the study area within 3 years. The study was approved by the Institutional Review Boards at the University of Pittsburgh and the University of Tennessee Health Science Center, and informed consent was obtained from all participants.

In the cross-sectional analyses, we included 2123 participants (48.2% male, 36.8% black), representing 69.0% of baseline participants. There were 2405 participants with a 2000-2001 clinic examination; 187 were deceased, 88 had a home visit, 233 had a telephone follow-up, 49 had a proxy interview only, 9 withdrew, and 104 missed the examination. We excluded participants missing serum B12 levels (n=98) at the 2000-2001 visit or the Digit Symbol Substitution Test [DSST] (n=184) at the 2001-2002 examination. Of the 2123 participants with data from the initial visit, 1095 participants had a 2007-2008 clinic examination; 492 were deceased, 120 had a home visit, 210 had a telephone follow-up, 129 had a proxy interview only, 9 withdrew, and 68 missed the examination, 43 did not have serum B12 levels and 19 did not have DSST scores. Thus, we included 1033 participants (47.9% male, 31.4% black) in the longitudinal analyses who had vitamin B12 levels at both the 2000-01 and 2007-08 visits and DSST scores at the 2001-02 and 2007-08 visits, representing 33.6% of baseline participants.

4.3.2 B12 assays

Vitamin B12 levels (using 300 ul sera stored at -70˚C until testing) were analyzed at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, University of Vermont, by a competitive immunoassay on the ADVIA Centaur (Bayer HealthCare, LLC) using direct chemiluminescent technology for the 2000-01 sample and using a Roche Elecsys 2010 chemical analyzer (Roche Diagnostics GmbH) for the 2007-08 sample. The Pearson correlation coefficient between these

two assays was 0.98 ($p<.01$) (Vogeser 2007). The normal range was 72 to 1427 pmol/L, which was determined from 272 serum samples. The assay CV ranges from 4% to 10% and a 6.7% CV was observed for the 5% of the sample blinded for quality control.

For the 2000-2001 sample, if serum B12 was <260 pmol/L, serum methylmalonic acid (MMA), total homocysteine (Hcy), serum 2-methylcitrate (2-MCA), and cystathionine were tested. Vials from the same blood draw for vitamin B12 were shipped to the University of Colorado Health Sciences Center for the metabolite assays and were analyzed by capillary gas chromatography-mass spectrometry (Stabler 1999). The normal ranges were 73-271 nmol/L for MMA, 5.4-13.9 µmol/L for Hcy, 60-228 nmol/L for 2-MCA, and 44-342 nmol/L for cystathionine.

Low vitamin B12 was defined as <260 pmol/L, and normal as≥260 pmol/L (Tucker 2005). Categories of 7-year change in vitamin B12 levels include: 1) Stay Normal; 2) Low to Normal; 3) Normal to Low; 4) Stay Low.

4.3.3 Cognitive tests

The Digit Symbol Substitution Test assesses information processing speed and psychomotor performance, and requires incidental memory, perceptual organization, visuomotor coordination, and selective attention to filter out irrelevant information [e.g., symbols that look alike] (Wechsler 1981). The participant is given a key of numbers and symbols on the top of the page, and then must fill-in the symbol corresponding to each number in the test section below. The participant has 90 seconds to fill in each symbol sequentially, and the score is the number of correctly completed symbols. The DSST has a high test-retest reliability (Matarazzo 1984). DSST scores decline with the onset of dementia (Storandt 1989; Strauss 1986), and DSST is

highly correlated with the Modified Mini Mental State Examination [3MS] (ρ=.56; p<.01) (Rosano 2005).

Global cognitive function was measured with the Modified Mini-Mental State Examination at the 2001-02 and 2007-08 visits (Teng 1987). The 3MS assesses cognitive domains of orientation, registration, attention, recall, and language. Scores range from 0 to 100, with higher scores indicating better cognitive function.

4.3.4 Potential confounders

Potential confounders were identified if they were associated with vitamin B12 and cognitive function and were included as covariates in the multivariable analyses. Demographic, socioeconomic factors (i.e., education, family income) and health information including alcohol use were assessed at the 1997-1998 visit. Other variables were from the 2000-2001 visit unless otherwise indicated. Diabetes was defined as fasting glucose ≥ 126 mg/dl, hypoglycemic medication use, or self-reported physician diagnosis. Hypertension was defined as blood pressure > 140/90 mmHg, hypertensive medication use, or self-reported physician diagnosis. All other chronic health conditions (i.e., cerebrovascular disease, coronary heart disease, congestive heart failure, peripheral arterial disease) were assessed at the 1997-1998 visit by self-reported physician diagnosis. Medication (CNS medication use [benzodiazepine and opioid receptor agonists, antipsychotics, antidepressants]; niacin use; fibrate use; statin use, thyroid medication, metformin) by medication inventory (Hilmer 2009) and smoking status were assessed during the 1999-2000 visit. Vitamin B12 supplement use was determined by use of either a multivitamin or B12 supplement (e.g., oral or intramuscular injection) at the 1997-98, 1998-99, 1999-2000, 2001-02, 2002-03, and 2007-08 visits. Blood pressure, cholesterol, ankle-arm index (low: <0.9;

normal: 0.9-<1.3; high:≥1.3), physical activity from walking and stair-climbing (kcal/kg/wk), high cystatin-C (≥1 mg/L), body mass index (BMI) [calculated as weight (kg)/ height^2 (m^2)] and depressed mood, using the Center for Epidemiologic Studies Depression (CES-D) scale (Radloff 1977), were assessed at the 2000-2001 visit.

4.3.5 Statistical analyses

Differences in demographics, socioeconomic and lifestyle factors, body composition, physiological factors, medication and supplement use, and chronic health conditions were tested by change in vitamin B12 levels, using Pearson χ^2 tests or Fisher's exact test for categorical variables and ANOVA, Kruskal-Wallis tests, or t-tests for continuous variables.

Cross-sectional analyses were performed using linear regression with DSST scores as the dependent variable and low vitamin B12 as the independent variable. Longitudinal analyses were done with low vitamin B12 levels and change in B12 levels separately as the predictors, using 1) linear regression for rate of change (slope) for DSST scores over 6-years (Fiocco 2010) and 2) logistic regression for having >1 SD change from mean 6-year change in DSST scores (Inzitari 2007). The SD of mean change was used but the SD of 2001-02 DSST was similar.

The models were built progressively in order, adjusting for covariates: demographics, socioeconomic and lifestyle factors, body composition, diabetes, physiological factors, medication and supplement use, and chronic health conditions. Age, gender, race, clinic site, education, and CES-D scores were adjusted for in all models; other variables were removed if p>0.10. Sensitivity analysis was conducted excluding participants who crossed the vitamin B12 cutpoint of 260 pmol/L at the follow-up visit, but did have an appreciable change (e.g., <50 pmol/L). Also, sensitivity analysis was also performed for deficient B12 levels at the 2000-2001

exam, where deficient B12 was defined as B12 <260 pmol/L and MMA >271 nmol/L and MMA >2-methylcitrate (Stabler 1986). Interactions with gender, race or education were tested though none were found (all p>0.05). Multicollinearity for independent variables was assessed using the variance inflation factor (VIF); no VIF was > 2.

Threshold analysis was performed using restricted cubic spline regression with 5 knots to determine whether there is a threshold serum vitamin B12 level for DSST decline (Desquilbet 2010). The knots were placed at the 5^{th}, 25^{th}, 50^{th}, 75^{th} and 95^{th} percentiles of serum B12 levels. To determine whether the threshold levels were significant, slopes before and after the cutpoint were compared using fully adjusted regression models. All analyses were conducted with SAS, version 9.2 (SAS Institute Inc, Cary, NC).

Comparing participants who only had data from the initial visit to those with follow-up data, those with only an initial visit were older (76.9 ± 2.9 years vs. 76.0 ± 2.8 years; p<.0001), had lower DSST scores (30.5 ± 14.4 vs. 38.8 ± 13.2; p<.0001) and lower 3MS scores (88.3 ± 9.8 vs. 93.0 ± 6.2; p<.0001). However, there was no significant difference in the prevalence of low vitamin B12 levels.

4.4 RESULTS

More than three quarters (76.7%) of the participants had normal B12 levels at both times, while 8.8% had low B12 levels at both times. About 8% improved to normal B12 and 6.6% dropped to low B12 levels. Participants with low B12 levels at both times were more likely to be male and white compared to participants with normal B12 levels at both times [Table 15]. Those whose B12 level was initially normal and became low at follow-up were more likely to be current

smokers and consume more than 1 alcoholic beverage a day compared to those with normal B12 levels at both times. Those with low B12 levels at follow-up were more likely to have diabetes compared to participants who improved to normal B12 levels. Figure 6 illustrates B12 supplement use by change in B12. Participants with low B12 levels at the initial visit were less likely to be taking supplemental B12 compared to participants with normal B12 levels at both visits. Supplement use among those who improved to normal B12 levels was around 30% from 1997-98 through the 2002-03 visit and increased to 54.9% at the 2007-08 visit.

There were no differences in DSST scores at 2000-01 or decline in DSST scores by initial B12 level [Table 16]. Table 17 shows that participants who improved to normal B12 levels had lower DSST scores at both times compared to those with sustained normal B12 levels.

In the cross-sectional analysis, low B12 levels were not associated with DSST scores (β=-0.810; p=.23) after adjusting for age, sex, race, site, education, CES-D score, income, CNS medication use, and prevalent cerebrovascular disease. Education and income attenuated the association, by 43% and 31% respectively. B12 deficiency was also not associated with DSST scores.

For the longitudinal analyses, low initial B12 was associated with a greater rate of DSST decline over 6-years (β=-0.267; p=.04), adjusting for initial DSST scores, age, sex, race, site, education, CES-D scores, thyroid medication use, CNS medication use, and prevalent CHD. Those improving to normal B12 levels had a greater rate of decline compared to those with normal B12 levels at both times (β=-0.465; p=.01), after adjusting for the same covariates [Table 18].

We also examined the association of having a decline >1SD from mean change (>14 points). Low initial B12 was not significantly associated with having a decline >1SD from mean

change (OR [95%CI]: 1.75 [0.99,3.09]), after adjusting for initial DSST score, age, sex, race, site, education, CES-D score, alcohol use, BMI, DM, CNS medication use, and prevalent peripheral arterial disease. CNS medication use attenuated the association, by 8%, and the association became non-significant. Those whose B12 level improved to normal had greater odds of having a decline in DSST scores >1SD (OR [95%CI]: 2.49 [1.20, 5.18]), after adjusting for the covariates above [Figure 7]. Associations for annualized DSST decline and decline >1SD from mean change were similar after excluding participants who changed B12 categories, but without an appreciable change in overall vitamin B12 level.

Finally, adjusted threshold levels were evaluated for DSST decline. Figure 8 shows the threshold levels, using restricted cubic spline analyses, for annualized DSST change, and Figure 9 demonstrates the threshold effect for having >1SD change from mean change. A threshold serum B12 level of 410 pmol/L was found for both annualized change and >1SD change, and was significant after adjustment (p=.01).

4.5 DISCUSSION

We found that low vitamin B12 levels (<260 pmol/L), higher than the cutpoint for deficiency (<148 pmol/L), were associated with greater DSST decline in older adults. Importantly, even higher serum B12 levels (~410 pmol/L) may be needed for lower cognitive decline. This study is important because few studies have examined the relationship between vitamin B12 and information processing speed, either cross-sectionally or longitudinally. Furthermore, the relationship between vitamin B12 and cognition is not fully understood and findings have been inconsistent. In addition, DSST scores have been found to predict cardiovascular events [stroke,

heart disease] (Elkins 2005), dementia (Rapp 2005), death (Fried 1998; Pavlik 2003), white matter disease progression (Longstreth 2005), and are associated with gait speed (Inzitari 2007), ADL disability (Kuo 2006), and telomere length (Yaffe 2009).

In this study, those who improved to normal B12 levels had worse DSST scores at both times and had greater decline in DSST scores. This finding is counterintuitive, and it is likely a marker of who is being treated, an effect and not a cause. These participants may have been treated for low or deficient B12, possibly because of symptoms related to low B12. However, there was no association between vitamin B12 level and anemia, CES-D scores, anxiety, fatigue, or peripheral neuropathy symptoms, but we did not examine all symptoms related to B12 deficiency during the visits and symptoms can be vague and non-specific. There was a substantial increase in B12 supplement among this group (26.9% in 1999-2000 vs. 54.9% in 2007-08), with a mean increase in serum B12 level of 274.0 ± 301.0 pmol/L. Supplement use in this group was still low at the 2002-2003 visit (34.2%); however, we do not have supplement data in between 2002-03 and 2007-08. The data suggests that supplement use was low for 6 years and supplement use increased at the follow-up visit. Thus, low B12 levels may have been sustained for a while and levels may have only been normalized shortly before the 2007-08 visit. Clinical trials have shown there may be a time-limited window of less than a year when cognitive impairment, associated with low B12, may be reversed with vitamin B12 treatment (Abyad 2002; Martin 1992). It is also possible that the participants only improved their B12 levels a short time before the 2007-08 visit and cognitive decline had already occurred. It is very important to study the longitudinal association, because if we only performed a cross-sectional analysis, we would not have appreciated the decline was greater even if B12 levels improved and that it may be important to prevent initially low B12 levels because cognitive impairments may

not always be easily reversed after vitamin B12 deficits. Also, we found adjusting for initial DSST scores was important, as the initial DSST scores strongly influenced the parameter estimates in the multivariable results.

Previous longitudinal studies did not find an association between serum B12 levels and cognitive function (Clarke 2007; Kado 2005; Mooijaart 2005; Kang 2006). However, these studies all analyzed serum B12 as a continuous predictor and did not use cutpoints for low or deficient B12. Our results suggest that there is a critical level for vitamin B12 rather than a continuous association. In the MacArthur Study and in the Nurses' Health Study, none of the cognitive tests assessed processing speed or motor performance (Kado 2005; Kang 2006). The Leiden 85-Plus Study included a test of processing speed (letter digit coding test) but did not find an association with vitamin B12 cross-sectionally or longitudinally (Mooijaart 2005). While we also did not find a cross-sectional association, we did find low B12 to be associated with greater decline in processing speed. In the analyses, they excluded participants who had low MMSE scores, which may have biased the results.

Strengths of this study were that we had longitudinal data for both vitamin B12 levels and DSST scores in a large cohort of black and white older men and women. A commonly used cutpoint for low B12 levels was used in the analyses, rather than using a deficient cutpoint (<148 pmol/L) or B12 as a continuous variable (Tucker 2005). We also examined whether there was a critical threshold level of serum B12 associated with lower decline in processing speed. Finally, our participants are well-functioning, so we expected to see small changes in DSST scores, and in our analyses we had enough statistical power to detect even a small difference.

A limitation of this analysis could be the small number of participants improving to normal or declining to low vitamin B12 levels. We also could not assess if and when they

started vitamin B12 treatment because of low or deficient B12 levels or symptoms related to B12 deficiency and we did not have homocysteine levels for all participants at both timepoints. We had a healthy population, as evidenced by high median 3MS scores at both the 2001-02 (i.e., 95) and 2007-08 (i.e., 94) visits. Few participants had a score below 80, only 3.5% at the 2001-02 visit and 8.2% at the 2007-08 visit, which is associated with cognitive impairment (Kuller 1998).

In conclusion, low vitamin B12 levels may be associated with slower processing speed and greater decline. Normal vitamin B12 levels (~260 pmol/L) may be needed, and higher serum B12 levels (~410 pmol/L) may be optimal to prevent or slow cognitive decline. Low vitamin B12 levels may need to be treated as soon as possible, because of the potential that cognitive impairments may not always be easily reversed. Randomized clinical trials are needed to determine if improving B12 levels to an optimal level can reverse cognitive impairments associated with B12 deficits and how long after symptoms appear can damage be reversed. In addition, clinical trials are needed to determine if higher B12 levels (~410 pmol/L) can slow cognitive decline. B12 supplementation is easily available, adequately absorbed, and highly tolerated in older adults, and may help prevent cognitive impairment associated with low vitamin B12 levels.

4.6 TABLES AND FIGURES

Table 15. Descriptive characteristics by 7-year change in vitamin B12 (<260 pmol/L)

	Stay Normal (n=792)	Low to Normal (n=82)	Normal to Low (n=68)	Stay Low (n=91)	p-value
Age (years)	76.0 ± 2.8	76.2 ± 2.6	76.0 ± 3.0	76.2 ± 2.6	0.41
Male (%)	45.2	54.9	57.4	58.2[c]	0.02
Black (%)	33.2	23.2	32.4	22.0[c]	0.06
BMI (kg/m^2)	27.3 ± 4.5	26.9 ± 4.1	27.8 ± 4.3	27.4 ± 3.8	0.79
Education level, %					0.31
Less than high school	15.9	22.0	20.6	17.8	0.43
High school grad	31.6	32.9	26.5	40.0	0.30
Post-secondary	52.5	45.1	52.9	42.2	0.20
Household income, %					0.13
Less than 10K	8.8	0.0	6.6	11.8	0.03
10K to 25K	33.5	42.1	36.1	34.2	0.50
25K to 50K	38.0	40.8	39.3	27.6	0.30
More than 50K	19.7	17.1	18.0	26.3	0.48
CES-D score	5.6 ± 5.9	5.2 ± 5.5	5.9 ± 7.0	5.3 ± 5.2	0.22
Smoking status, %					0.004
Former	45.0	58.0[a]	56.1	46.1	0.06
Current	4.3	6.2	12.1[b]	5.6	0.04
Alcohol use, %					0.13
None in past year	18.3	21.3	16.7	12.2	0.44
<1 per week	21.7	22.5	31.8	24.4	0.29
1-7 per week	24.5	22.5	19.7	28.9	0.59
>1 per day	7.2	12.5	15.2[b]	8.9	0.07
Physical activity (kcal/kg/wk)	36.6 ± 55.5	34.5 ± 57.7	34.8 ± 40.2	32.5 ± 61.9	0.17
SBP (mmHg)	133.2 ± 18.6	135.2 ± 21.7	130.6 ± 17.4	136.0 ± 19.7	0.78
DBP (mmHg)	71.2 ± 10.1	71.7 ± 10.3	69.6 ± 10.4	72.1 ± 10.6	0.60
Ankle-arm index, %					0.36
Low	10.4	12.2	15.4	11.4	0.64
Stiffening	5.5	0.0[a]	6.2[d]	4.6	0.17
Total cholesterol (mg/dL)	193.1 ± 34.9	179.5 ± 25.0	192.7 ± 36.6	190.3 ± 37.7	0.19
Cystatin-C ≥1 mg/L (%)	38.9	50.6[a]	52.2[b]	47.2	0.02
Statin use (%)	22.2	23.5	22.4	13.3	0.26
Niacin use (%)	1.9	0.0	0.0	1.2	0.78
Fibrate use (%)	1.2	1.3	1.7	0.0	0.41
Thyroid meds (%)	13.7	12.4	14.9	12.2	0.67
CNS meds (%)	11.5	16.3	10.8	10.2	0.60
Diabetes status, %					0.19
Impaired fasting glucose	16.3	20.7	20.6	16.5	0.63
Diabetes	17.3	9.8	25.0[d]	20.9[e]	0.08
Metformin use (%)	17.0	25.0	29.4	22.2	0.50
Hypertension (%)	69.2	75.6	65.7	70.0	0.58
CBVD (%)	4.6	6.3	9.0	4.5	0.38
CHD (%)	13.7	16.7	11.3	23.8[c]	0.07
CHF (%)	0.4	0.0	1.5	0.0	0.35
PAD (%)	3.6	3.7	4.5	3.4	0.96
3MS score					
2001-2002	93.2 ± 6.4	93.3 ± 4.9	91.6 ± 6.5	92.9 ± 4.9	0.13
2007-2008	91.8 ± 8.0	90.7 ± 7.0	90.8 ± 8.7	91.3 ± 6.5	0.04

Table 15 continued

Hemoglobin (g/dL)	13.8 ± 1.3	13.6 ± 1.3	13.8 ± 1.4	14.0 ± 1.1[e]	0.16
Mean corpuscular volume (fL)	91.0 ± 5.0	91.6 ± 4.7	89.7 ± 5.7[d]	89.7 ± 4.9[c,e]	0.01
Anemia	11.1	18.3	10.6	10.1	0.25
Numbness in legs/ft	28.6	22.2	26.5	25.3	0.61
Aching/burning pain in legs/ft	16.3	20.7	14.7	18.7	0.68
Anxiety	15.2	18.3	25.4[b]	14.4	0.16
Fatigue	18.6	19.5	25.0	18.7	0.61
B12 level 2000-01 (pmol/L)	481.7 ± 205.4	221.4 ± 33.9[a]	337.7 ± 62.5[b,d]	210.6 ± 33.8[c,e,f]	<0.0001
Change in B12 (pmol/L)	61.9 ± 227.3	274.0 ± 301.0[a]	-121.9 ± 70.9[b,d]	-17.9 ± 44.8[c,e,f]	<0.0001

Pairwise: p<0.05 for a: Low to Normal vs. Stay Normal; b: Normal to Low vs. Stay Normal; c: Stay Low vs. Stay Normal; d: Normal to Low vs. Low to Normal; e: Stay Low vs. Low to Normal; f: Stay Low vs. Normal to Low (low B12 <260pmol/L; normal B12 ≥260pmol/L)

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; CNS: central nervous system; CBVD: cerebrovascular disease; CHD: coronary heart disease; CHF: congestive heart failure; PAD: peripheral arterial disease; CES-D: Center for Epidemiologic Studies Depression; 3MS: Modified Mini-Mental Examination

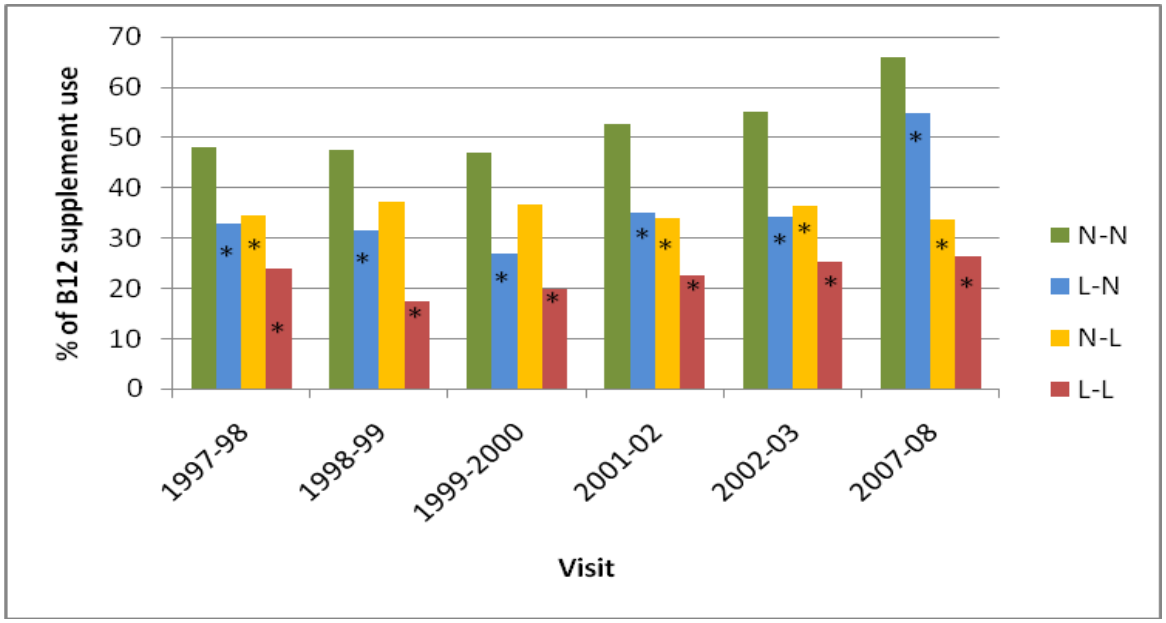

Figure 6. B12 Supplement Use by Change in B12 from 2000-01 to 2007-08

L=Low <260pmol/L; N=Normal B12 ≥260pmol/L
*p < 0.05 compared to stay normal B12

Table 16. DSST by 2000-01 vitamin B12 level

	Normal (n=860)	Low (n=173)	p-value
2001-02 DSST*	39.1 ± 13.4	37.4 ± 11.7	0.11
Rate of change for DSST*	-0.62 ± 1.61	-0.77 ± 1.60	0.28
>1SD from mean change, %	9.4	12.7	0.19

Low B12 <260 pmol/L; Normal B12 ≥260pmol/L; 1 SD from mean change = 14 points
*Age-adjusted means

Table 17. DSST by 7-year change in vitamin B12 level

	Stay Normal (n=792)	Low to Normal (n=82)	Normal to Low (n=68)	Stay Low (n=91)	p-value
1997-98 DSST*	41.0 ± 13.5	37.1 ± 11.6[a]	39.7 ± 13.8	38.9 ± 13.2	0.04
2001-02 DSST*	39.2 ± 13.3	36.2 ± 11.8[a]	37.6 ± 14.7	38.4 ± 11.6	0.20
2007-08 DSST*	35.4 ± 13.4	30.8 ± 11.2[a]	34.4 ± 14.2	34.5 ± 13.0	0.02
Rate of change for DSST*	-0.63 ± 1.59	-0.90 ± 1.50	-0.53 ± 1.82	-0.65 ± 1.68	0.47
>1SD from mean change, %	9.5	14.6	8.8	11.0	0.49

Pairwise: p<0.05 for a: Low to Normal vs. Stay Normal
Low B12 <260pmol/L; Normal B12 ≥260pmol/L; 1 SD from mean change = 14 points
*Age-adjusted means

Table 18. Multivariable linear regression models for change in B12 and rate of DSST change

	Low to Normal		Normal to Low		Stay Low	
	β	p	β	p	β	p
Model 1: unadjusted	-0.284	0.13	0.099	0.63	-0.020	0.91
Model 2: adj. for 2001-02 DSST	-0.416	**0.02**	0.029	0.88	-0.061	0.71
Model 3: adj. for above + demographics	-0.430	**0.01**	0.050	0.79	-0.063	0.70
Model 4: fully adjusted*	-0.465	**0.01**	-0.030	0.87	-0.085	0.62

Reference: stay normal B12; low <260pmol/L; normal B12 ≥260pmol/L
*Adjusted for 2001-02 DSST, age, sex, race, site, education, CES-D score, thyroid medication use, CNS medication use, prevalent CHD

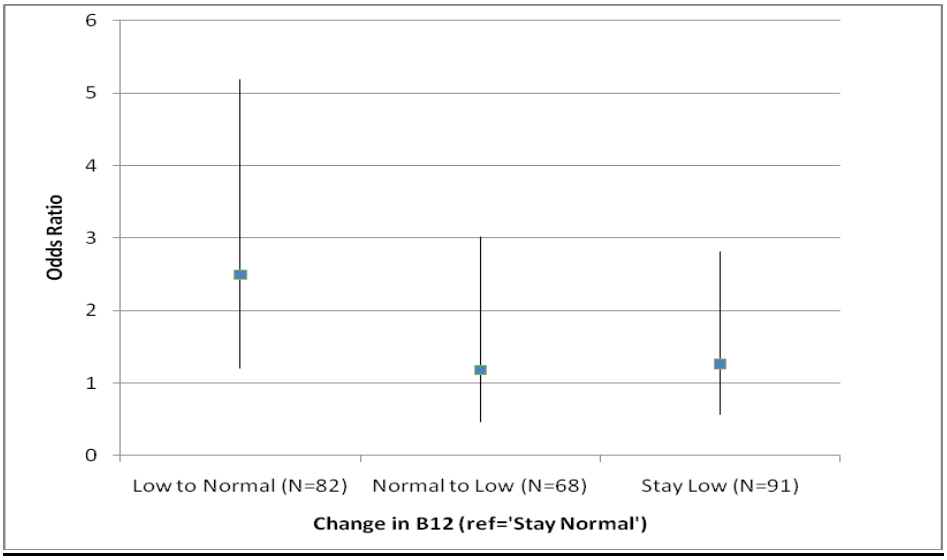

Figure 7. Multivariable logistic regression models for DSST >1SD from mean change

Low <260pmol/L; normal B12 ≥260pmol/L; 1 SD from mean change = 14 points
*Adjusted for 2001-02 DSST, age, sex, race, site, education level, CES-D score, alcohol use, BMI. DM, CNS med use, prevalent PAD

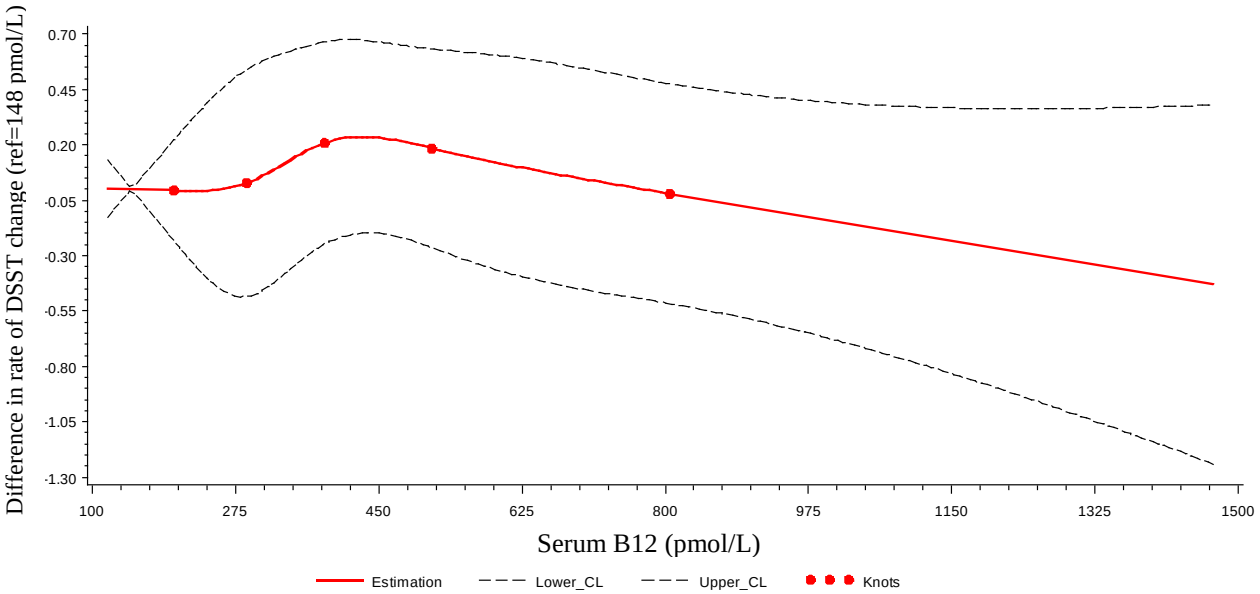

Figure 8. Threshold of serum B12 level for rate of DSST change

*Adjusted for 2001-02 DSST, age, sex, race, site, education, CES-D score, thyroid medication use, CNS medication use, prevalent CHD

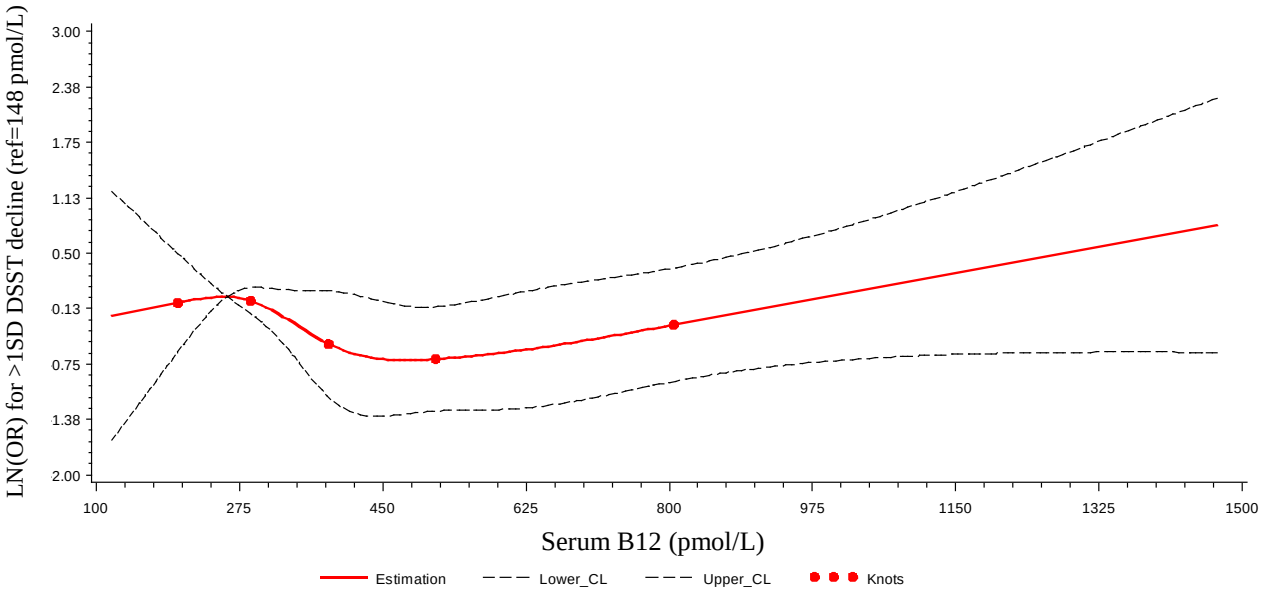

Figure 9. Threshold of serum B12 level for having >1SD change from mean change

*Adjusted for 2001-02 DSST, age, sex, race, site, education level, CES-D score, alcohol use, BMI. DM, CNS med use, prevalent PAD

5.0 DISCUSSION

5.1 SUMMARY OF FINDINGS

The results of these analyses indicate that vitamin B12 levels are related to neurological function and that higher B12 levels than currently clinical guidelines are important for optimal neurological function in old age. We found that sustained high homocysteine was associated with lower nerve conduction amplitude and greater insensitivity to 4g monofilament touch in older adults from the InCHIANTI Study. These findings were important, because it is the first study, to our knowledge, that has examined the association between homocysteine and peripheral nerve function, cross-sectionally or longitudinally, and that looked at both change in B12 and homocysteine and change in peripheral nerve function. In the Health ABC Study, poor B12 (either low or deficient B12) was associated with greater insensitivity to 1.4g monofilament detection and lower nerve conduction velocity. We found there was a threshold effect for serum B12 level of 390 pmol/L, which is higher than current clinical cutpoints. Thus, higher levels of vitamin B12 may be needed for optimal peripheral nerve function. This is the first study that has critically examined whether there is a threshold effect of vitamin B12 on peripheral nerve function. Finally, we found low B12 was associated with greater decline in information processing speed. Interestingly, those improving to normal B12 levels had greater decline. This may have been because they were treated with B12 supplementation after symptoms of B12

deficiency were noticed. Studies have suggested there may be a limited time period when cognitive impairments may be reversed with B12 treatment (Abyad 2002; Martin 1992). Therefore, it is important to maintain optimal B12 levels and prevent B12 levels from degrading to low or deficient. We found serum B12 levels of 410 pmol/L were significantly associated with lower decline in processing speed, which was similar to the threshold observed for peripheral nerve function.

5.2 PUBLIC HEALTH SIGNIFICANCE

There is a high prevalence of low vitamin B12 levels and high homocysteine levels in older adults. Vitamin B12 is essential for neurological function. It is important to evaluate vitamin B12 levels and neurological function in older adults after the mandatory folic acid fortification in the United States, because large doses of folic acid may cause B12 deficiency to go unnoticed, and irreversible neurological function may occur (Varela-Moreiras 2009). We found low vitamin B12 levels and high homocysteine levels were associated with worse peripheral sensory and motor nerve function in older adults. Low vitamin B12 levels were associated with greater decline in information processing speed. Vitamin B12 levels need to remain at optimal levels, because anemia may not be present and once levels become low, neurological impairments may not always be reversible. Higher serum B12 levels (~400 pmol/L), above current clinical cutpoints, may be needed for optimal neurological function. Worse peripheral nerve function may lead to impaired physical function and disability in older adults (Resnick 2000; Resnick 2001; Strotmeyer 2008; Strotmeyer 2009). Early detection and treatment of low vitamin B12 levels are critical to prevent neurological damage. Vitamin B12 supplementation is efficacious

and due to many different causes of malabsorption in older adults, it may be an appropriate treatment to prevent decline in peripheral nerve and cognitive function in older adults, potentially reducing disability and falls. Randomized clinical trials are needed to determine if higher vitamin B12 levels are associated with better neurological function. The Flour Fortification Initiative is currently advocating the fortification of vitamin B12 in grains. In order to push fortification, evidence is needed for the suffering and disability that low/deficient B12 levels may cause. Specifically, B12 fortification would be aimed to increase B12 levels in pregnant women, children, and older adults (Allen 2009). More research is needed on the benefits of fortification on functional outcomes (e.g., cognitive and physical). Our findings show that low B12 (not only deficient B12) is associated with worse sensory and motor peripheral nerve function and greater cognitive decline, and much higher serum B12 levels, than the current clinical deficient level, may be optimal for neurological function. This is very important, because even in the Health ABC Study, a healthy population of older adults, only 1% had serum B12 levels below 148 pmol/L (clinically deficient), 17% had low serum B12 levels (< 260 pmol/L), while nearly half (48%) had levels below 400 pmol/L. Since higher B12 levels may be therapeutic and B12 levels can be effectively increased with B12 in the crystalline form (Eussen 2005, Allen 2009), this dissertation supports the evidence that B12 fortification in flour may be critical to help improve vitamin B12 levels in older adults and prevent neurological declines.

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