ASSOCIATION BETWEEN FOLATE, VITAMIN B12 AND COGNITIVE PERFORMANCE IN DEMENTED ELDERLY

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Dementia is prevalent among elderly people. As the world population ages, it is projected that the number of people affected by dementia may triple in the next 50 years. Over the last two decades, research has focused on identifying potentially modifiable risk factors in development and progression of dementia, such as vitamin B12 and folate. Results concerning the effects of low folate and vitamin B12 on cognitive performance are mixed. The main objective of the present study was to investigate the effects of vitamin deficiency on cognitive functioning in a clinical sample of elderly individuals with cognitive problems using a comprehensive neuropsychological assessment. A retrospective chart-review was performed on the 102 records of patients from the Geriatrics Clinic at the University of North Texas Health Science Center who presented with cognitive deficits. Charts were reviewed to obtain data on vitamin supplementation, vitamin status, history of chronic conditions and other biochemical data. The available database was used to obtain data on neuropsychological assessment. The study demonstrated mild association between vitamin B12 and folate status and cognitive deficits. There appeared to be a higher cut-off level that is above the traditionally used levels for vitamin B12 and folate deficiency concentrations at which cognitive deficits became more pronounced. Clinical applications, limitations and suggestions for future research were discussed.
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INTRODUCTION

Dementia is prevalent among elderly people. It is predicted that it would place a heavy burden on the health care system in the near future. As the world population ages, it is projected that the number of people affected by dementia may triple in the next 50 years (Hebert, Scherr, Bienias, Bennett & Evans, 2003). Cognitive function was identified as a major determinant of the quality of life in the elderly population (Rosenberg & Miller, 1992). Alzheimer disease and vascular dementia are two most common types of dementia. Initially treated as etiologically different disorders, the two subtypes of dementia have been shown to share pathological features. Vascular factors play a significant role in Alzheimer disease. Over the last two decades, research has focused on identifying potentially modifiable risk factors in development and progression of dementia. This research has identified dietary related factors such as the B-vitamin group including folate, vitamin B12 and vitamin B6, and related metabolites such as homocysteine (Hcy) as important in cognitive functioning. Framingham Heart Study found high prevalence of inadequate B vitamin status in elderly population (Selhub, Jacques, Wilson, Rush, & Rosenberg, 1993), with 30% of individuals having folate deficiency and 20-25% having vitamin B12 deficiency. Understanding the nature of the association between cognitive functioning and dietary intake and plasma/serum concentrations of vitamins in aging elderly is exceedingly important. Much of the research has focused on the relationship between vitamin B deficiency and raised Hcy levels.

Homocysteine and Cognitive Dysfunction

There has been extensive research showing increased levels of Hcy in patients with Alzheimer's dementia when compared to normal controls (Clarke et al., 1998; McCaddon, Davies, Hudson, Tandy & Cattell, 1998; Lehman, Gottfried & Regland, 1999; Stewart,
Asonganyi & Sherwood, 2002). A large Baltimore Memory Study (n = 1,140) found an association between Hcy and cognition (Schafer et al., 2005). Garcia and Zanibbi (2004) in their review on Hcy and cognition concluded that elevated Hcy levels are associated with vitamin B deficiency, cognitive decline and dementia.

High plasma Hcy level was found to be a strong, independent risk factor for developing vascular dementia and Alzheimer disease (Seshadri et al., 2002). In a large prospective study (n = 1092), dementia-free individuals who were in the highest quartile of serum Hcy had a relative risk of 1.9 for developing Alzheimer disease 8 years later (Seshadri et al.). Raised plasma Hcy concentration greater than 14 mmol/L doubled the risk of developing Alzheimer's dementia (Seshadri et al.). McCaddon et al. (1998) found that elevated levels of Hcy not only related to decreased cognitive function, but also predicted the rate of cognitive decline in healthy elderly over 5 years (McCaddon et al., 1998; McCaddon et al., 2001). In a large prospective (n = 1,241) study of healthy people over 60, Dufouil, Alperovitch, Ducros and Tzourio (2003) found that individuals with Hcy levels greater than 15 mmol/L had 2.8 times the odds of cognitive decline of at 4-yr follow-up, compared with those with Hcy levels below 10 mmol/L.

Many studies have found a relationship between elevated Hcy levels and decreased cognitive functioning in non-demented elderly (Budge, de Jager, Hogervorts & Smith, 2002; Morris, Jacques, Rosenberg & Selhub, 2001; Riggs, Spiro, Tucker & Rush, 1996). Hcy levels correlate with cognitive function even in healthy elderly and within the conventionally considered normal range of Hcy values. Prins et al. (2002) identified Hcy levels of about 14 mmol/L as threshold above which adverse cognitive performance was noted in non-demented elderly. Studies show that a variety of cognitive functions are compromised in healthy elderly with high Hcy levels (Selhub, Bagley, Miller & Rosenberg, 2000).
Homocysteine and Neuropathology

Mechanisms of Hcy action are still widely debated. It has been suggested (e.g., Linderman et al., 2000) that neurocognitive decline in the elderly may be mediated by cerebrovascular changes and related elevated concentrations of Hcy. Elevated plasma Hcy is associated with an increased risk of stroke, coronary heart disease (e.g., Bots, Launer, Lindemans, Hofman & Grobbee, 1997), vascular diseases (Homocysteine collaboration, 2002; Klerk et al., 2002), atherosclerosis (Bots et al., 1997; Refsum et al., 1998). For example, Miller, Mungas, Reed and Jagust (2002) found that elevated Hcy levels are more common among patients with vascular disease than among those with Alzheimer's disease. It has been argued (Kalmijn et al., 1999) that oxidized forms of Hcy are neurotoxic. Others argue (Rosenberg & Miller, 1992) that Hcy acts via disturbance of methylation of DNA, neurotransmitters and phospholipids.

Regardless of its mechanisms, elevated Hcy levels are associated with cerebral and medial temporal lobe atrophy, and white matter damage. Clarke et al. (1998) found that elevated Hcy levels were associated with atrophy of the medial temporal lobe. Also, higher levels of Hcy in individuals with confirmed Alzheimer disease have been associated with a more rapid atrophy rate of that region (Clarke et al.).

In normally-aging elderly, high Hcy levels are associated with atrophy of hippocampus (den Heijer et al., 2003; Williams, Pereira, Budge and Bradley, 2002). Williams et al. (2002) found that even in a group \( n = 156 \) of normal elderly without clinical memory problems hippocampal width was inversely related to the level of Hcy. Similarly, den Heijer et al. (2003) found elevated levels of Hcy related to cortical and hippocampal atrophy in non-demented elderly. These finding are important, as the progressive order of brain atrophy in Alzheimer's
disease begins precisely with the atrophy in medial-temporal lobe in entorhinal cortex, which is part of the parahippocampal gyrus (Smith, 2002). Atrophy in this area is identified in people with Mild Cognitive Impairment (Smith).

The level of Hcy was found to be associated with white matter abnormalities (Fassbender et al., 1999). Hcy level was related to silent brain infarcts and white matter lesions in the Rotterdam Scan Study (Vermeer et al., 2002). It was found as levels of Hcy increase, so does the risk of silent brain infarcts, the severity of periventricular white matter lesions and the extent of the subcortical white matter lesions. Individuals with Hcy levels in the upper normal range between 9.9 and 13.7 mmol/L had a significantly greater risk of silent brain infarcts and severe white matter lesions, compared with those with Hcy levels were below 8.5 mmol/L, considered the lower normal range. Individuals with above the normal range levels of Hcy had the highest odds ratio for all MRI-detected abnormalities (Vermeer et al.). Sachdev, Valenzuela, Wand, Looi and Brodaty (2002) showed that Hcy was a significant determinant of the brain atrophy, measured by ventricular-brain ratio, but that it did not contribute to white matter abnormalities or cortical atrophy.

**Homocysteine - Vitamin Connection**

Homocysteine (Hcy) is an amino acid that is produced in the metabolism of methionine with the help of vitamin B6, vitamin B12, and folic acid. The possibility that lowering Hcy levels can be achieved through correction of nutritional deficiencies in folate and vitamin B12 has encouraged research of association between these two vitamins and cognitive function. Folate (folic acid or folacin) and vitamin B12 (cobalamin) are two vitamins known to be related to brain functioning (Martin, 1988). Both are biological determinants of Hcy, and are consistently found to be negatively correlated with Hcy levels (e.g., Duthie et al., 2002; Rodriguez et al., 2006). Hcy
is derived from dietary methionine as a result of deficiency in B6, B12, folic acid, or TMG (trimethylglycine) (Holford, 2004). It is toxic and needs to be removed rapidly. Vitamin B12 serves as co-factor in the process of recycling Hcy back to methionine. Hcy can also be converted to cysteine with the help of vitamin B6. Ubbink, Vermaak, van der Merwe & Becker (1993) reported that plasma Hcy serves as a marker for status of vitamin B12, folate, and vitamin B6. Concentrations of folate and vitamin B12 are positively correlated. Folic acid and vitamin B12 are water-soluble vitamins. Vitamin B12 is found in foods of animal origin (meat, eggs, milk) and folic acid is found in green leafy vegetables, fruits, yeast, and organ meats, such as liver (Brewster, 1984; Hassing et al., 1999).

Multiple factors can be responsible for vitamin deficiency in elderly population, including malabsorption due to atrophic gastritis, inadequate dietary intake, increased excretion due to renal impairment, and increased vitamin requirements secondary to hematological changes (Hassing et al., 1999). Higher prevalence of atrophic gastritis with advancing age had been cited as the most frequent cause of vitamin deficiency (Baik & Russell, 1999). Atrophic gastritis results in low secretion of acid-pepsin by gastric mucosa, and hypochlorhydria. The former reduces vitamin B12 release from food proteins, and the later increases growth of bacteria in the stomach and small intestines that interfere with B12 absorption (Baik & Russell). It should be noted that absorption of vitamin B12 in the crystalline form (i.e., vitamins supplements and fortified foods) is unaffected by the presence of atrophic gastritis (Baik & Russell). Therefore, the Food and Nutrition Board has recommended that elderly receive their B12 vitamin via vitamins and fortified products. Inadequate dietary intake hypothesis that proposes that individuals with cognitive deficits exhibit poor dietary/nutritional habits that lead to vitamin deficiency is generally unsupported by research, as other nutritional deficiencies which would be
expected in individuals with cognitive deficits are not found (Levitt & Karlinsky, 1992). Vitamin deficiency has been suspected to be a consequence of dementia-related neuropathological changes, such as cell death and alterations in cell functions leading to poor vitamin absorption and utilization (Levitt & Karlinsky). Vitamin deficiency is then viewed as a marker of irreversible disease progression, and changes in nutritional status through supplementation would be expected to have no positive effects on cognition. Calvaresi and Bryan (2001) suggested two metabolic pathways in which B vitamins affect functioning of the CNS: a direct, by the process of hypomethylation that inhibits methylation reactions involving proteins, membrane phospholipids, DNA, neurotransmitters and melatonin, and an indirect, by influencing Hcy concentrations.

Folate and Cognitive Dysfunction

Folic acid plays a significant role in the function of the nervous system. Maternal folate deficiency during pregnancy is a risk factor for developing neural tube defects in a child (Reynolds, 2002b). In neonates, infants, children and adolescents, malfunctions of folate metabolism are associated with multiple deficits, including developmental delay, cognitive deterioration, motor and gait abnormalities, behavioral and psychiatric disturbances (Reynolds, 2002b). A review of the research literature by Alpert and Fava (1997) concluded that folate plays a critical role in metabolic pathways of the brain and its deficiency is associated with multiple neuropsychiatric disorders, including depression and dementia. In geriatric population, folate deficiency has high prevalence (Selhub et al., 1993) and is related to dementia, depression, apathy, withdrawal, and lack of motivation (Reynolds, 2002a).

One of the original studies relating folate concentrations to cognitive function was by Sneath, Chanarin, Hodkinson, McPherson and Reynolds (1973). It was reported that in a group
of geriatric patients, patients with dementia had lower levels of folate than patients with other
diagnosis (Sneath et al.). Low folate levels have been found in patients with Alzheimer disease,
vascular dementia and people with cognitive deficits (Clarke et al., 1998; Hassing et al., 1999;
Wahlin et al., 1996). Folate levels were found to be lower in patients with histologically
confirmed AD and vascular dementia compared to healthy controls (Clarke et al., 1998; Ebly,
Schaefer, Campbell & Hogan, 1998). In a recent study, Ravaglia et al. (2005) concluded that low
serum folate and elevated plasma Hcy were independent predictors of development of Alzheimer
disease. Low folate levels are associated with greater risk of developing Alzheimer's disease
(Clarke et al., 1998). In a 3-year prospective community-based study in Sweden, part of the
Kungsholmen Project (n = 370) Wang et al. (2001) explored the relationship between low serum
levels of folate and vitamin B12, separately and combined in the development of AD in non-
demented individuals over 75. Wang et al. found that compared to individuals with normal levels
in both vitamins, those with low folate ( < 10 and < 12 nmol/L) had double the risk of
developing AD over 3 years. Bottiglieri et al. (2001) found that individuals with AD had lowest
plasma folate levels, compared to individuals with other types of dementia . Many other studies
suggest that low folate levels are associated with dementia (e.g., Ebly, Schaefer, Campbell &
Hogan, 1998; Hassing, Wahlin, Winblad, & Backman, 1999; Lindeman, Romero & Koehler,
lower serum folate and B12 levels have been reported to have poorer cognition (Bernard,
Nakonezhny & Kashner, 1998; La Rue et al., 1997; Riggs, Spiro, Tucker & Rush, 1996; van
Goor, Woiski, Lagaay, Meinders & Tak, 1995). Generally, a positive relation has been found
between cognitive performance and intake or blood level of folate.
Debate regarding the role of folate in cognitive function in the elderly is ongoing. Recent reviews on relationship between Hcy levels, folate, B vitamins and cognitive function have come to different conclusions. A review by Calwaresi and Bryan (2001) of 8 cross-sectional, 2 longitudinal, and 4 experimental studies arrived at the conclusion that there was good evidence to suggest that there is an association between B vitamins and cognitive performance/decline. On the other hand, a critical evaluation of three case control and three cohort studies by Ellinson, Thomas and Patterson (2004) concluded that there is no consistent relationship between cognition and serum folate levels.

There are multiple studies reporting on benefits of and fortification of foods with folic acid on cognition. In a placebo-controlled study by Botez, Botez, Leveille, Bielmann and Cadotte (1979) four months of folic acid supplementation in individuals with folate deficiency, mild cognitive impairment and depressed mood improved memory and constructional ability. Rapin, Le Poncin and Grebyl (1988) found that elderly individuals with low levels of folate, impaired memory and depression significantly improved visuomotor performance, visuospatial memory, logical reasoning, associative memory and activities of daily living after four months of folinic acid supplementation. In a double-blind, placebo-controlled study of the elderly with memory complaints, two months of folate supplementation resulted in improved memory and attention (Fioravanti et al., 1997). Interestingly, cognitive improvement was greatest among individuals with greater folate deficiency (Fioravanti et al.).

**Folate and Neuropathology**

A very limited number of studies explored association between low folate and neuropathology. Botez, Fontaine, Botez and Bachevalier (1977) related folate-deficiency to atrophy of neocortex assessed by computerized transaxial tomography. They found individuals
with chronic folate deficiency to have impaired cognitive function and performance on neuropsychological tests. Cognitive performance improved after 6-12 month of supplementation with folic acid (Botez et al., 1977). In a study of 30 elderly Catholic nuns with the same environmental/nutritional background, Snowdon et al. (2000) examined 18 nutrients, lipoproteins and nutritional markers, and found only serum folate levels were negatively related to cerebral atrophy of the frontal, temporal, and parietal lobes at autopsy. This relationship was even stronger in a subset of 15 nuns with significant numbers of Alzheimer disease lesions. Clark et al. (1998) found that higher plasma Hcy and lower folate levels at baseline were associated with greater atrophy of the medial temporal lobe and a more rapid disease progression over three years in 76 individuals with neuropathologically confirmed Alzheimer disease. Of interest, Hcy levels were found to be stable over a 3-year follow-up period.

It was hypothesized that folate relates to poor cognitive function through its ability to reduce levels of Hcy (Ubbink, Vermaak, van der Merwe & Becker, 1993), which in turn is related to vascular problems. Results by Bottiglieri et al. (2001) suggest that low levels of folate may cause dementia by elevating Hcy levels. Elevated Hcy is consistently associated with low folate in the population of elderly with dementia (e.g., Bottiglieri et al.). It was also suggested that folate can be implicated in non-vascular mechanisms involved in decreased cognitive function through its role in neurotransmitter metabolism (Ordonez, 1979).

**Folate and Specific Cognitive Deficits**

**MMSE and Global Cognitive Functioning**

Despite the relative insensitivity of Mini-Mental Status Exam (MMSE; Folstein, Folstein, & McHugh, 1975), as a measure of global cognitive functioning, most research supports relationship between MMSE and folate. Stewart et al. (2002) and Duthie et al. (2002) found
serum folate was positively correlated with scores on MMSE, while Hcy was negatively correlated with scores on MMSE in healthy elderly. Leblhuber et al. (2000) also reported serum folate to be positively correlated with MMSE scores in a group of patients with probable Alzheimer's and probable vascular dementia ($n = 31$). Lindeman et al. (2000) in a large study of a randomly selected sample of the elderly comparing serum vitamin B12, C and folate concentration and multiple measures of cognitive function found a significant association between low serum folate concentrations and lower cognitive performance on MMSE. On the other hand, a longitudinal study by Tucker, Qiao, Scott, Rosenberg and Spiro (2005) found no association between MMSE and any biochemical measures assessed, including folate among 321 aging men.

**Memory**

There is substantial support for association between low folate and memory deficits (Duthie et al., 2002; Goodwin, Goodwin & Garry, 1983; Lindeman et al., 2000; Morris et al., 2001; Nurk et al., 2005; Teunissen et al., 2003). In the population-based ($n = 2189$) Hordaland Homocysteine study, Nurk et al. (2005) reported that elderly individuals with episodic memory deficit measured by the Kendrick Object Learning Test (KOLT; Kendrick, 1985) had higher Hcy and lower folate levels, compared with those without memory deficit. Morris et al. (2001) found that elderly in the upper half of the folate distribution performed better on a paragraph delayed-recall test, a measure of short-term verbal memory than those in the lower half of the distribution. Teunissen et al. (2003) concluded that folic acid concentration correlated with the delayed recall test in normal aging elderly. The Kungsholmen ageing and dementia study reported low serum vitamin B12 levels and especially low serum folate levels to be related to poor episodic memory in 321 healthy individuals in the community ages 71 to 101 (Wang et al., 2003).
Low folic acid was also associated with poorer episodic memory in a study by Wahlin, Hill, Winblad and Backman (1996). Lindeman et al. (2000) found significant association between low serum folate concentrations and the Fuld Object Memory test (FOME) in a randomly selected sample of the elderly. Duthie et al. (2002) in a cohort study of 186 healthy elderly found that folic acid was positively correlated with Auditory Verbal Learning Test (AVLT), a measure of verbal learning and memory.

Hassing et al. (1999) assessed the effects of low levels of vitamin B12 and folic acid, separately and combined on memory in a sample of 71 healthy very old adults by administering a battery of memory tests comprising face recognition, immediate and delayed free word recall, delayed word recognition and object recall (using the FOME). Low folic acid concentration (below 13 nmol/L) had negative effects on immediate and delayed word recall, and object recall. Interestingly, correlational analysis revealed only one significant correlation between folic acid and immediate word recall. Hassing et al. found that low levels of folic levels have a negative effect on long-term retrieval, but not short-term retrieval processes. Also, whereas folic levels were related to free recall, they were not related to recognition tasks. Hassing et al. concluded that low levels of folic acid in very old age may influence encoding and retrieval processes, while leaving primary memory unaffected.

Among studies that found no folate-memory relationship are studies by La Rue et al. (1997), Riggs et al. (1996), and Tucker et al. (1990). La Rue et al. (1997) found no significant correlation between folate levels and scores on Logical Memory and Visual Reproduction subtests of the Wechsler Memory scale, measures of verbal and non-verbal memory in healthy elderly. Using Word List Memory test adapted from CERAD, Backward Digit Span (WAIS-R), Activity Memory test, and Pattern Memory (NES2) to assess memory function, Riggs et al.
(1996) found no relationship between fasting plasma concentrations of folate and performance on these tests in 70 healthy elderly men. All three studies involved correlational or regressional design. Hassing et al. (1999) attempted to explain these negative results by arguing that the relationship between folate and memory is non-linear. Hence, there might be a critical point in folate levels below which deficiency negatively impacts memory.

**Visuospatial Abilities**

With a few exceptions, research literature generally supports relationship between folate concentrations and visuospatial abilities. In a study of a cohort of individuals born in 1936 \(n = 148\), Duthie et al. (2002) found folate to be positively correlated with WAIS Block Design (a measure of visuospatial organization) performance. Relationship between spatial difficulties and folate levels is further supported by the Boston Veterans Affairs Normative Aging Study (Riggs et al., 1996). Riggs et al. explored the relationship between fasting plasma concentrations of Hcy, Vitamin B12, vitamin B6 and folate and cognitive function in 70 healthy elderly men. Cognitive functioning was assessed using the Consortium to Establish a Registry for Alzheimer's Disease Neuropsychological Assessment Battery (CERAD), the Pattern Comparison, Continuous Performance Test and Pattern Memory from Neurobehavioral Evaluation System (NES2), Vocabulary and Backward Digit Span subtests of WAIS-R, and Paper Folding. Among the variety of cognitive functions assessed, low serum folate (\(< 3 \text{mu g/L}\)) was associated only with poor spatial copying abilities, as assessed by the CERAD Construction Praxis test.

In the VA Normative Aging Study, Tucker, Qiao, Scott, Rosenberg & Spiro (2005) examined the relation between baseline fasting plasma Hcy, folate, vitamin B12, and vitamin B6 and cognitive decline in 321 aging men. Cognitive testing included a measure of working memory (backward digit span subtest from WAIS-R), recall (word list memory test computer-
adapted from CERAD), language (verbal fluency test from CERAD), spatial copying (constructional praxis from CERAD) and the MMSE, and was repeated three years later on average. As in their research group's earlier cross-sectional study (Riggs et al., 1996), Tucker et al. found spatial copying measure most sensitive to low B vitamin and high Hcy. Decline in constructional praxis was associated with plasma Hcy, folate, vitamins B6 and B12. Tucker et al. also found that men with folate levels < 20 nmol/L were more likely to lose spatial ability than were those with folate levels > 30 nmol/L, who on average, had no apparent loss.

Contrary to the findings by Duthie et al. (2002), Riggs et al. (1996) and Tucker et al. (2005), La Rue et al. (1997) found no relationship between folate and visuospatial skills, as measured by Rey-Osterrieth Complex Figure Test and Visual Reproduction subtest of the Wechsler Memory Scale in a group of healthy normally-aging elderly. No relationship was found between low serum folate below 11 nmol/L (5ng/mL) and performance on the test of visuoconstruction, the Clock Face test in a randomly selected sample of elderly (Lindeman et al., 2000).

**Abstract Reasoning/Executive Functions**

In a community-based study of 260 healthy elderly, part of larger New Mexico Aging Process Study that started in 1979, low folate levels were associated with impaired abstract thinking, measured by the Halstead-Reitan Categories Test (Goodwin, Goodwin & Garry, 1983). It's important to note that participants were well-educated, health-conscious, financially secure, and highly motivated, as reported by researchers, and hence do not constitute a random sample of older adults. La Rue et al. (1997) followed the sample of healthy normally-aging older adults (n = 137) studied by Goodwin et a. (1983), and also found a significant correlation between fasting plasma and erythrocyte folate levels and abstract reasoning, measured the Abstraction
Scale from the Shipley-Hartford Intelligence Test. Folate intake was also related to abstract reasoning (La Rue et al.).

The New Mexico Aging Process Study explored differences in cognition among elderly individuals taking vitamin supplements, including vitamin B12 and folate, and those not taking any (Abou-Saleh & Coppen, 1989). Authors reported that although no difference was detected on tests of memory and visual perception, individuals taking supplements performed better on more complex tests of cognition involving visuospatial skills, abstraction and non-verbal memory.

Attention and Information Processing

Duthie et al. (2002) administered a battery of neuropsychological tests to 334 healthy elderly from two cohorts (1921 and 1936). The battery included MMSE, NART (National Adult Reading Test), RPM (Raven's Progressive Matrices), AVLT (Auditory Verbal Learning Test) and WAIS subtests. Duthie et al. (2002) found a positive correlation between low folate and Digit Symbol scores, a measure of information processing speed in 1921 birth cohort. Low folate was associated with poor performance on Symbol Search (measure of processing speed and scanning) in a recent study by Elias et al. (2006). Folate was related to Scanning and Tracking composite (Trails A and B, Digit Symbol Substitution, Symbol Search) in dementia-free population (Elias et al., 2006). Lindeman et al. (2000) found low serum folate related to lower performance on the Digits Forward (measure of attention and immediate memory) among randomly selected elderly. Elderly individuals with low folate levels also performed worse on one of the two Color Trails tests (measure of psychomotor speed and cognitive flexibility) (Lindeman et al., 2000). On the other hand, Tucker et al. (2005) discovered no association between working memory (Backward Digit Span subtest of WAIS-R) and folate in aging men.
Vitamin B12 and Cognitive Dysfunction

Vitamin B12 plays a role in myelin formation in the nervous system (Brewster, 1984). Vitamin B12 deficiency has a variety of clinical manifestations, including megaloblastic anemia and neurological abnormalities, from paresthesias to spastic ataxia.

The prevalence of vitamin B12 deficiency among elderly population ranges from 3% to 60% (Andres et al., 2004; Lewerin et al., 2005; Selhub et al., 1993; Baik & Russell, 1999). Ellison, Thomas and Patterson (2004) cite malabsorption of vitamin B12 as the main cause of vitamin B12 deficiency in the elderly. Deficiency in vitamin B12 can negatively affect cognition by leading to increased Hcy levels (e.g., Miller et al., 2002).

There is evidence that even moderately low and subclinical vitamin B levels can be related to cognitive impairment (Selhub, Bagley, Miller & Rosenberg, 2000). Vitamin B12 levels were found to be lower in patients with histologically confirmed AD and vascular dementia compared to healthy controls (e.g., Clarke et al., 1998). Bottilieri et al. (2001) found that individuals with vascular dementia had the highest levels of plasma Hcy and the lowest plasma vitamin B12, compared to individuals with dementia Alzheimer's type (AD), Lewy body dementia (LBD) and frontotemporal dementia (FTD). Wang et al. (2001) found that among non-demented individuals over 75, individuals with low vitamin B12 levels (defined as < 151 and < 251 pmol/L) had more than doubled the risk of developing AD over 3 years compared to those with normal levels. Individuals with B12 levels under 151 pmol/L had a 60% increase in AD incidence, whereas individuals with B12 levels under 251 pmol/L had even higher, 80% increase in AD incidence.

Published research shows that association between vitamin B12 and cognitive dysfunction in the elderly population is weaker than that found for folate or Hcy (Basun,
Fratiglioni & Winblad, 1994; Clarke et al., 1998; Joosten et al., 1993; Lehmann, Gottfries & Regland, 1999; Lindeman et al., 2000; Ravagli et al., 2003; Snowdon et al., 2000; Teunissen et al., 2003; Wahlin, Wahlin, Winblad & Backman, 2001; Wang et al., 2001). Studies assessing relationship between low concentration of vitamin B12 and folate and cognitive performance, consistently lend support to the argument that folate is more important to cognition, than vitamin B12 (e.g., Lindman et al., 2000). Serum vitamin B12, which is the most commonly used screen for vitamin deficiency is considered a non-sensitive and non-specific measure of vitamin status, since normal serum B12 levels can be present concurrent with metabolically deficient B12 levels (Green & Kinsella, 1995; Stabler, 1995; van Goor et al., 1995). Vitamin B12 metabolite determinants, methylmalonic acid (MMA) and serum Hcy are reported to be more sensitive markers than serum vitamin B12 deficiency (e.g., Allen, Stabler, Savage, & Lindenbaum, 1990; Ellison, Thomas & Patterson, 2004). The serum MMA is highly specific to vitamin B12 metabolism, and represents the current preferred indicator of vitamin B12 status (Baik & Russell, 1999). In his literature review, Wang (2002) reported that whereas there is disagreement in studies on whether deficiency or low concentration of vitamin B12 is more common in individuals with Alzheimer dementia, compared to healthy controls, studies using metabolic indicators for vitamin B12 deficiency, show a more clear connection.

Randomized placebo-controlled vitamin B12 supplementation trials have produced inconclusive results about the beneficial effects of vitamin supplementation on cognitive function in later life. Whereas studies show that B12 supplementation successfully improves biochemical vitamin deficiency level, there is no evidence of improved cognitive function (Eussen et al., 2005; Eussen et al., 2006; Seal, Metz, Flicker, & Melny, 2002). Eussen et al.
(2006) warned that it is uncertain whether improvements in vitamin B12 in peripheral blood reflect vitamin status in cerebrospinal fluid and neurons of the central nervous system.

**Vitamin B12 and Neuropathology**

Research on neuropathology associated with vitamin B12 deficiency is scarce. In the nun study, Snowdon et al. (2000) found no relationship between vitamin B12 concentration and brain atrophy. Clarke et al. (1998) reported that there was a trend towards greater disease progression, as assessed by medial temporal lobe thickness, among individuals with lower vitamin B12 levels.

**Vitamin B12 and Specific Cognitive Deficits**

**MMSE and Global Cognitive Functioning**

Research on the relationship between vitamin B12 and global cognitive functioning is conflicting. In a recent study by Tucker et al. (2005) MMSE, a measure of global cognitive functioning was not related to vitamin B12 levels in aging men. A critical evaluation of three case control and three cohort studies by Ellison, Thomas and Patterson (2004) concluded that there is no consistent relationship between scores on the MMSE and serum vitamin B12 or serum folate levels. Low vitamin B12 serum levels were not related to MMSE scores in healthy elderly. Other studies support the relationship between MMSE and vitamin B12 levels. Healthy elderly with higher MMSE scores had higher serum and erythrocyte concentrations, compared to those with lower scores (Ortega et al., 1996). Stewart et al. (2002) and Duthie et al. (2002) found that serum vitamin B12 was positively correlated with scores on MMSE. Impairments in global cognitive function in dementia-free individuals with low B12 were reported by Bernard et al. (1998). In a large group of elderly meeting criteria for probable or possible AD, individuals with low vitamin B12 level (< 200 pg/ml) showed greater global cognitive deficits on two measures of global cognitive functioning (MMSE and Blessed Dementia Scale) (Whyte et al., 2002).
Early studies identified a vitamin B12-memory connection. Goodwin et al. (1983) reported that individuals with low levels of vitamin B12 showed memory deficits, assessed by a revised version of the Wechsler Memory Test. Vitamin B12 was positively related to visual and verbal immediate and delayed recall in dementia-free and stroke-free community-dwelling persons aged 60 or more years (Elias et al., 2005). Most of the other studies failed to find this association. Among healthy elderly, low vitamin B12 concentrations alone had no impact on episodic memory (Wahlin et al., 1996). However, individuals with low levels of both vitamin B12 and folate performed significantly worse, than those with normal levels of both. Hassing, Wahlin, Winblad and Backman (1999) did not find low concentration of vitamin B12 (below 180 pmol/L) to be related to any aspect of memory assessed, including face recognition, immediate and delayed free word recall, delayed word recognition and object recall in a sample of 71 healthy very old adults. Bernard, Nakonezny and Kashner (1998) and Jelicic, Jonker and Deeg (2001) found no difference in memory tests performance among subjects with normal and low levels of serum vitamin B12 and folate. La Rue et al. (1997) did not find an association between plasma vitamin B12 concentration and verbal and nonverbal memory. Individuals with low serum vitamin B12 levels did not perform worse on the FOME test of memory. Verbal learning and memory were not related to vitamin B12 status in the study by Teunissen et al. (2003). In the population-based (n = 2,189) Hordaland Homocysteine study, Nurk et al. (2005) found that vitamin B12 concentrations were not related to memory function. In a 5-year prospective cohort study, part of the Bronx Longitudinal Aging Study Crystal et al. (1994) annually measured serum B12 and administered a battery of tests, including two tests assessing memory, the Blessed Test of Information, Memory and Concentration (BIMC), and the Fuld Object Memory Evaluation
(FOMEx) to 410 physically healthy and cognitively intact elderly aged 75 to 85 years. Crystal et al. found that low serum B12 < 150 pmol/L concentration was not related to deficit in memory. Also, individuals with low B12 concentrations did not develop dementia, AD or other type, more frequently compared with those with high B12 concentrations (Crystal et al.). Crystal et al. concluded that low B12 was not a marker or a cause for dementia.

Visuospatial abilities

Among healthy elderly men, low vitamin B12 (< 200 ng/L) was associated with poor spatial copying abilities, as assessed by the CERAD Construction Praxis test (Riggs et al.). La Rue et al. (1997) did not detect relationship between B12 and visuospatial skills. Visuoconstruction ability, assessed by the Clock Face was not related to vitamin B12 levels in health elderly (Lindeman et al., 2000).

Abstract Reasoning/Executive Functions

Although, La Rue et al. (1997) did not find relationship between plasma vitamin B12 concentration and performance on neuropsychological tests assessing verbal and nonverbal memory and visuospatial skills, dietary intake of vitamin B12 was related to abstract reasoning. Goodwin et al. (1983) found poor performance on a test of abstract thinking and problem solving, the Halstead-Reitan Category Test in a group of healthy elderly with low B12 concentrations. Vitamin B12 was found to be positively related to performance on the Similarities in dementia-free population (Elias et al., 2006). In healthy elderly, low levels of vitamin B12 were associated with poorer scores on tests of abstract reasoning and selective attention (specifically, the Stroop Color-Word Test) (Bohnen, Jolles, & Degenaar, 1992).
No association between vitamin B12 level and performance on Backward Digit Span subtest of WAIS-R, a measure of working memory was found by Tucker et al. (2005). Performance on the Digits Forward subtest of WAIS-R, a measure of attention and immediate memory was also not related to B12 status in healthy elderly from another study (Lindeman et al., 2000). Teunissen et al. (2003) found no correlations between vitamin B12 concentration and performance on a test assessing cognitive speed, attention and information processing.

Interrelationship Between Folate and Vitamin B12

Wahlin et al. (1996) and Hassling et al. (1999) examined concomitant effects of deficiency in both vitamin B12 and folate. Wahlin et al. (1996) found that whereas deficiency in vitamin B12 was not related to deficit in episodic memory, deficiency in folic acid was associated with deficit in episodic memory performance. This association was even more pronounced in people with concomitant deficiency in folic acid and vitamin B12 (Wahlin et al.). Hassling et al. (1999) did not find combined effect of low vitamin B12 (below 180 pmol/L) and low folate (13 nmol/L) on memory tasks, and concluded that that folic acid is a more sensitive marker of cognitive deficits than vitamin B12. No interaction effects between folic acid and vitamin B12 were found.

Explaining Mixed Results

One explanation for mixed research findings are different exclusion criteria used in different studies. Statistical design used (correlational or group comparison) can also explain differences in research findings. Finally, different cut-off levels selected for identifying vitamin deficiencies contribute to equivocal findings. Currently, there are no age-adjusted norms for vitamin B12 and folate or universal cut-off values identifying deficiency. Hassing et al. (1999)
suggests that memory may be sensitive to vitamin deficiency in older rather than younger elderly individuals. Hence, the same level of vitamin deficiency may have a differential effect on cognition, depending on individual's age.

Although most studies measure serum folate levels, Hassing et al. (1999) and Bottiglieri (1996) discussed erythrocyte folate (or red-blood cell folate) as a more reliable indicator of body folate storage. Brewster (1984) also cited erythrocyte folate as the best accepted laboratory deficiency index. Whereas serum levels indicate folate levels during the past few days, erythrocyte levels reflect levels during the past few months (Hassing et al.). General laboratory threshold of erythrocyte folate deficiency is 280 ng/ml RBC (referenced by Quest Laboratories). Threshold of serum folate deficiency typically used in research is 14 nmol/L (Ortega et al., 1996) or 3 ng/mL. Other cut-offs for low serum folate used are 17 nmol/L (Clarke et al., 1998), 10 nmol/L and 12 nmol/L (Wang et al., 2001).

Reference range used for serum vitamin B12 is 200-1100 pg/ml (referenced by Quest Laboratories). There is no consensus about what level of serum vitamin B12 constitutes deficiency. Researchers classified elderly with serum vitamin B12 concentration below 200 pmol/L (Clarke et al., 1998), 250 pmol/L (Lindenbaum et al., 1994; Pennypacker et al., 1992), and 110 pmol/L (Crystal et al., 1994) as having deficiency. Wang et al. (2001) used two cut-off points for serum vitamin B12, 151 and 251 pmol/L. They based 150 pmol/L on research suggesting that 90% of older individuals have tissue deficiency below this point (Wang et al.). According to Carmel et al. (1999), choosing cut-off of 258 pmol/L eliminates majority of individuals with tissue deficiency. Since reports on level of B12 that constitutes vitamin deficiency are inconsistent, we base the choice of two cut-offs on Wang et al.'s advise. We chose two cut-off points to define low vitamin B12, 151 pmol/L and 251 pmol/L. The lower cut-off
point is likely to include majority of vitamin-deficiency cases, whereas the higher cut-off point is likely to include probable vitamin-deficiency cases.

Another confounding variable is whether blood samples used were fasting or non-fasting. Fasting serum folate and vitamin B12 are generally considered to be a more accurate measure of vitamin status (e.g., Ellison, Thomas & Patterson, 2004). Yet, many studies use random non-fasting blood samples.

Linderman et al. (2000) failed to find association between low B12 concentrations (200 pg/mL and 300 pg/mL) and cognitive function. They explained this by the inability of the serum B12 concentration to identify B12 deficiency. Linderman et al. stated that low serum B12 is not necessarily indicative of vitamin deficiency, and a 'normal' concentration of B12 does not rule out a deficiency. Metz et al. (1996) found that using conventional practice of setting serum B12 concentration at 150 pmol/L as the low limit for identifying B12 deficiency in the elderly missed 90% of patients with tissue B12 deficiency. Pennypacker et al. (1992) reported that using 147 pmol/L as a cut-off point missed approximately 50% of B12-deficient individuals. Linderman et al. (2000) suggested using Hcy as one of the alternatives to identifying B12 deficiency.

Objectives of the Present Study

In the view of mixed results concerning the effects of low folic acid and vitamin B12 on cognitive performance, we designed this study to clarify the relationship between vitamin B12 and folate levels and cognitive performance in a geriatric population. One of the main limitations of the published literature is a large proportion of studies with a small sample size that limits their power. Also, many studies assessed neurocognitive function using a screening tool, like MMSE or narrow neuropsychological batteries that fail to provide a comprehensive assessment. Many studies have been population-based, assessing healthy elderly individuals.
The purpose of the present study is to obtain further evidence concerning the effects of vitamin deficiency on cognitive functioning in a large clinical sample of elderly individuals with cognitive problems using a comprehensive neuropsychological assessment. Study intends to determine which cognitive tests are most sensitive to low concentrations of vitamin B12 and folate. In addition, the study aims to identify a cut-off for vitamin B12 and folate concentrations below which cognitive deficits become more pronounced in geriatric population. Another objective of the study is to assess whether folate and vitamin B12 concentrations can differentiate between types of dementia (AD, vascular dementia, mixed dementia and mild cognitive impairment) and severity of dementia. Based on involved neuropathology of folate deficiency and previous findings, we hypothesized that folate will be positively related to performance on tests of memory, abstract reasoning and executive functions, and attention and information processing. It was hypothesized that vitamin B12 concentration will be positively associated with performance on tests of abstract reasoning and executive function. We expected lower vitamin B12 and folate concentrations to be related to greater severity of dementia.
METHOD

A retrospective chart-review was performed on the records of patients from the Geriatrics Clinic at the University of North Texas Health Science Center who presented with cognitive deficits, and were referred for a neuropsychological assessment by their physicians. The available database containing data for these neuropsychological assessments was used to guide the medical chart selection procedure.

Neuropsychological Assessment

The neuropsychological database contained records of the neuropsychological and affective assessment of 319 patients. Assessments were performed between January 2002 and November 2005 by a licensed psychologist, post-doctoral fellows or trained student clinicians, and evaluated multiple domains of cognitive functioning. The assessment required approximately two hours to complete, and included a clinical interview, typically completed in collaboration with a patient's caregiver, and a comprehensive battery of neuropsychological tests. Neuropsychological test measures included in the battery were the following: the Hooper Visual Organization Test (HVOT; Hooper, 1958); Wechsler Memory Scale-Third Edition (WMS-III; Wechsler, 1997) subtests - Logical Memory I, Logical Memory II, Logical Memory Recognition, Visual Reproduction I, Visual Reproduction II, Visual Reproduction Recognition, Letter-Number Sequencing, Mental Control, Digit Span, and Spatial Span; Consortium to Establish a Registry for Alzheimer's Disease (CERAD; Morris et al., 1989) subtests - Verbal Fluency, Boston Naming Test, Word List Memory Task, Word List Recall Task, Word List Recognition Task, and Constructional Praxis; Behavioral Dyscontrol Scale; Trailmaking Test A (TMT-A; Army Individual Test Battery, 1944); Trailmaking Test B (TMT-B; Army Individual Test Battery, 1944); and Clock Drawing Test (CDT). Geriatric Depression Scale (GDS; Yesavage et
al., 1983) was used to measure depression. Because of performance difficulties, some individuals were unable to complete the full neuropsychological assessment. Mean substitution was used to estimate missing values in the analysis for variables missing less than 10% of data points.

Record Selection Procedure

Due to the retrospective nature of the study, there were inevitable problems with missing data. Medical charts were available and reviewed for 156 patients. Several patients had repeated neuropsychological testing. Only one record was used per patient to ensure that no patient had an unequal weight in the analysis. If a patient had two or three pairs of neuropsychological and laboratory evaluations, the record with shortest time lapse between the two evaluations was chosen for the analysis. Majority of patient records had vitamin B measures included in their laboratory work. However, no lab work for vitamin B12 or folate was ordered by patients' physicians in 25 cases (9%). One hundred two cases with lab work performed within 90 days before or after neuropsychological testing were retained for the analysis. The mean number of days between lab work and neuropsychological evaluation was 27.44 (SD = 19.63) days, ranging from 0 to 83 days. Medical charts were reviewed to obtain information on vitamin intake. Vitamin intake was recorded for the date closest to the lab work. With the exception of a few cases, most vitamin intake records were obtained for the day of the blood work.

Physiological/Biochemical Data

Medical charts were reviewed to obtain the following medical and biochemical information: serum vitamin B12, serum or RBC folate, creatinine, blood urea nitrogen (BUN), cholesterol (high density lipoprotein (HDL), low density lipoprotein (LDL), and total cholesterol), thyroid-stimulating hormone (TSH), history of hypertension, history of hypercholesterolemia, and history of hypothyroidism. Vitamin supplementation data was
obtained for vitamin B12 and folate. For vitamin B12, participants were included in the vitamin supplementation group if they received vitamin B12 injections, or took vitamin B12, B-complex, or multivitamin supplements orally. For folate, participants were included in the vitamin supplementation group if they orally took folic acid, B-complex, or multivitamin supplements. Medical charts of the 102 individuals included in the analysis, indicated that 57 patients (55.9%) received vitamin B12, 59 patients (57.8%) received folic acid, and 55 patients (53.9%) received both B12 and folic acid supplements.

Participant Characteristics

The mean age of the participants studied was 77.86 ($SD = 6.46$) years, ranging from 65 to 94 years. There were 74 females and 28 males. Education was quantified in years of education. Among 102 records, 19 individuals (18.6%) were missing information about their education level. The mean number of years of education for the 83 available cases was 13.19 ($SD = 3.99$), ranging from 3 to 23 years. No information on the ethnicity of participants was collected but this sample group was predominantly Caucasian. Majority of participants were living in the community. Mean MMSE total score for the entire sample was 23.35 ($SD = 4.18$), with scores ranging from 10 to 30.
RESULTS

Prevalence of Chronic Conditions

All analyses were performed using SPSS computer software version 11.5. There was a high prevalence of chronic conditions present in the studied sample. Information on prevalence of chronic conditions of hypothyroidism, hypercholesterolemia, and hypertension for each diagnostic group is presented in Table 1. Chi-Square analysis did not find significant differences between the five diagnostic categories in the prevalence of chronic conditions of hypothyroidism ($\chi^2(4) = 2.24, p = .69$), hypercholesterolemia ($\chi^2(4) = 9.2, p = .06$), and hypertension $\chi^2(4) = 7.99, p = .09$).

Vitamin B12 and Cognitive Function

For the 101 subjects with available serum vitamin B12, mean value was 619.30 pg/mL ($SD = 385.92$) with values ranging from 179 pg/mL to 1960 pg/mL. The distribution of B12 values was positively skewed, with the median of 529 pg/ml. Two participants were identified as univariate outliers based on extremely high serum vitamin B12 measures ($z > 3.29$). Analysis conducted with and without outliers yielded similar findings. Results will be reported for the dataset that includes outliers.

Calculating the group of demented patients as a whole ($n = 101$), no significant correlation was found between the MMSE scores and serum B12 values, indicating no direct relationship between the degree of general cognitive impairment and vitamin B12 status. Serum vitamin B12 correlated negatively with digit span total score ($r = -.22, p = .027$), and positively with LMII retention percentage ($r = .24, p = .016$). Serum vitamin B12 was also positively correlated with the total score on the GDS ($r = .20, p = .042$). Comparisons were made between individuals with deficient and normal vitamin B12 nutritional status on their performance on the
tests measuring neurocognitive functions. Due to the lack of consensus in the literature as to what comprises vitamin B12 deficiency level, three cut-off values derived from prior research were explored in the analysis (300 pg/mL, 250 pg/mL, and 200 pg/mL). Thirteen subjects (12.7%) had B12 levels under 300 pg/mL, six subjects (5.9%) had B12 levels under 250 pg/mL, and three subjects had B12 levels under 200 pg/mL (3.0%). Hence, depending on the cut-off point for vitamin B12 deficiency, the prevalence of vitamin B12 deficiency in this sample ranged from 3.0% to 60%. This is on the lower end of estimated 7% to 60% vitamin B12 deficiency prevalence in elderly population cited in literature (Andres et al., 2004; Baik & Russell, 1999; Selhub et al., 1993). Thirteen individuals (12.9%) had vitamin B12 levels that exceeded the upper limit of the recommended reference range (200-1100 pg/mL). Independent samples t-test found no significant difference in age, education level, symptom severity level (based on the MMSE scores) or performance on neuropsychological tests between individuals with conventionally considered low (under 300 pg/ml) and normal vitamin B12 serum levels (over 300 pg/mL). Similarly, no significant difference was found when B12 levels of 200 pg/mL and 250 pg/mL were used as cut-off points to determine deficiency. Based on the Mann-Whitney test, vitamin B12 deficient individuals did not differ from non-deficient individuals in terms of diagnosis or diagnosis severity. Analysis of data without outliers (based on vitamin B12 values) produced similar results.

Comparisons of groups with different diagnoses on vitamin B12 using univariate ANOVA yielded a significant difference, $F(4, 96) = 4.503, p < .01$. Vitamin B12 values for different diagnostic groups are presented in Table 2.

Scheffe post hoc analysis indicated that individuals diagnosed with Alzheimer's disease had significantly lower mean vitamin B12 values ($M = 500.6, SD = 225$) than individuals who
received 'other' diagnosis ($M = 956, SD = 584.3$), $p = .02$. Patients diagnosed with MCI ($M = 504.5, SD = 242.5$) also had lower vitamin B12 values compared to patients diagnosed with 'other' dementia ($M = 956, SD = 584.3$), $p = .05$. The difference in vitamin B12 values between AD and VaD was approaching level of statistical significance, with patients with AD demonstrating lower serum B12. These two diagnostic groups (AD and VaD) were not different in severity level (Mann-Whitney test, $z = -.64$, $p = .52$). A similar trend was found between patients with AD and mixed dementia. Similar severity levels were observed between the two diagnostic groups (Mann-Whitney test, $z = -41$, $p = .68$), with a trend towards lower vitamin B12 levels in individuals with AD compared to individuals with mixed dementia. It is of interest that the two diagnostic categories with the lowest serum vitamin B12 were AD and MCI. These two groups significantly differed in severity level, based on the results of the Mann-Whitney test ($z = -3.61$, $p < .001$), with Alzheimer's patient on average having mild-moderate severity, and patient with MCI on average having mild severity.

When median split was performed on vitamin B12 laboratory measures, patients in the upper half of the B12 distribution (above 529 pg/mL) performed better on LMII than patients in the lower half of the distribution (below 529 pg/mL). Patients with B12 values in the upper half of the distribution on average recalled 8.73 ($SD = 7.83$) ideas, compared to 5.54 ($SD = 6.72$) ideas recalled by those in the lower half of the distribution, $t(100) = -2.21$, $p = .03$. Corresponding difference between LMII scaled scores with mean of 6.98 ($SD = 3.48$) and mean of 5.38 ($SD = 3.21$) was also significant, $t(100) = -2.42$, $p = .02$. The LMII retention percentage was significantly higher in patients with B12 values in the upper half of the distribution ($M = 29.91, SD = 30.97$), compared to patients with B12 values in the lower half of the distribution ($M = 29.91, SD = 30.97$), compared to patients with B12 values in the lower half of the distribution ($M$
Hence, both correlational and group comparison analyses suggested relationship between higher serum B12 level and performance on the LMII.

To explore composition of the two groups formed by the median split based on vitamin B12 values, non-parametric Mann-Whitney test was used. The two groups were not different in terms of diagnostic category \((z = -1.71, p = .09)\), diagnosis severity \((z = -0.76, p = .45)\), age \((z = -0.1, p = .92)\), or years of education \((z = -1.06, p = .29)\). Significant difference was found between the two groups only in vitamin supplementation \((z = -2.2, p = .03)\). Individuals with vitamin B12 above the medial level had greater vitamin supplementation as a group. Not surprisingly, vitamin B12 supplement users had higher serum B12 concentrations \((M = 723.37, SD = 450.03)\) compared to nonusers \((M = 484.48, SD = 222.82), p < .01.\)

Folate and Cognitive Functioning

Two types of folate were found in patient's medical charts, red blood cell and serum folate. Of the 99 available records of folate, 35 records were of red blood cell folate and 64 records were of serum folate. Used laboratory defined low serum folate as less than 3.4 ng/mL, borderline folate as ranging between 3.4 ng/mL and 5.4 ng/mL, and normal folate as greater than 5.4 ng/mL. For 64 subjects, mean serum folate was 20.44 ng/mL \((SD = 5.02)\) with values ranging from 2.3 ng/mL to 24 ng/mL. It should be noted that the clinical laboratory used set 24 ng/mL as the upper limit for serum folate, and any measure greater than 24 ng/mL is specified as such. Thirty-four subjects had serum folate levels specified as greater than 24 ng/mL. Hence, the true mean serum folate value is underestimated. Based on the laboratory reference range for folate, only one individual with 2.3 ng/mL serum folate fell in the folate deficiency range. The traditional level of distinguishing serum folate deficiency is 3 ng/mL (or 3 µg/L) (Lovati et al., 2007). Lindeman et al. (2000) and Lindenbaum et al. (1994) in the Framingham study identified
serum folate deficiency as 5 ng/mL. However, even using the later criteria only one individual in our sample had serum folate levels in the deficiency range, resulting in the less than 1% prevalence rate for folate deficiency.

Normal laboratory reference range for red blood cell folate was defined as greater than 280 mg/mL. Borderline red blood cell folate level was between 130 and 279 mg/mL, and low was less than 129 mg/mL. For 35 subjects, mean red blood cell folate level was 604.66 mg/mL ($SD = 170.78$) with values ranging from 364 mg/mL to 1000 mg/mL. Due to the lack of clinically defined folate deficiency in this sample, no comparison of folate deficient and non-deficient individuals could be performed.

Calculating the group of demented patients as a whole ($n = 35$), no significant correlation was found between the MMSE scores and red blood cell folate level, indicating no direct relationship between the severity of general cognitive impairment and folate status ($p = .68$). Similarly, no significant correlation was found between the severity of general cognitive impairment and folate status in patients with recorded serum folate level ($n = 64$). Red blood cell folate positively correlated with the LMI slope ($r = .40, p = .02$) and negatively correlated with spatial span backward total score ($r = -.34, p = .04$). Serum folate did not correlated with any test of cognitive function. This can be attributed to the ceiling effect in serum folate measurement and the resulting peak of folate values at 24 ng/mL.

Median split was performed separately on serum folate and red blood cell folate laboratory measures before the two groups were combined. Median value for red blood cell folate of 593 mg/mL was used in the analysis. Of the 64 patients with recorded serum folate, 34 (53.1%) patients had folate measures over 24 ng/mL. All 34 individuals were assigned to the upper half of the folate distribution. This accounted for the unequal sample sizes between the
upper half \((n = 52)\) and the lower half \((n = 47)\) of the folate distribution for combined serum and red blood cell folate measures. Patients in the upper half of the folate distribution performed better on the BNT \((M = 13.53, SD = 1.47)\), compared to patients in the lower half of the folate distribution \((M = 12.75, SD = 2.18, t(97) = -2.1, p = .04)\). In the analysis restricted to 65 individuals with Trails B scores, patients in the upper half of the folate distribution obtained significantly higher scaled scores on Trails B \((M = 4.1, SD = 3.1)\), than patient in the lower half of the folate distribution \((M = 2.57, SD = 2.37, t(63) = -2.19, p = .032)\).

Next, the composition of the two groups formed by the median split based on folate values was explored. Mann-Whitney test yielded no significant difference between the two groups in diagnostic category \((z = -1.14, p = .26)\), age \((z = -1.16, p = .25)\), or years of education \((z = -1.46, p = .14)\). Significant difference was found between the two groups in diagnosis severity \((z = -2.1, p = .04)\), and vitamin supplementation \((z = -3.27, p < .01)\). Individuals with folate values below the median had greater severity of cognitive impairment and lower folate supplementation (27%), compared to individuals with folate values above the median who demonstrated less severe cognitive impairments and higher folate supplementation (60%).

**Renal Insufficiency**

Impaired renal function (based on elevated serum creatinine) was highly prevalent in this sample (19.6%). For 91 available values, a significant positive correlation was found between the BUN and vitamin B12 values, Pearson \(r = .26, p = .01\). Correlation between creatinine and vitamin B12 was approaching significance, Pearson's \(r = .2, p = .05\). Compared to individuals with vitamin B12 values below the median \((M = 17.22, SD = 6.79)\), individuals with vitamin B12 values above the median \((M = 21.02, SD = 6.6)\) had significantly higher BUN values \((p = .01)\), signifying relatively higher level of renal insufficiency. Correlational analysis between red blood
cell folate and creatinine also yielded a positive correlation, Pearson's $r = .39$, $p = .03$ ($n = 31$). Due to the ceiling of serum folate values it was not possible to explore the relationship between serum folate and creatinine.

To clarify relationship between renal insufficiency and vitamin B12 values, individuals with excessive vitamin B12 values (above 1100 pg/mL) were separated and compared to the rest of the sample on creatinine and BUN values. Thirteen individuals (12.9%) had vitamin B12 levels that exceeded the recommended reference range. These individuals had higher creatinine values ($M = 1.27$, $SD = .37$) compared to those whose vitamin B12 values were below the high cut-off of the reference range ($M = 1.07$, $SD = .32$), with the difference approaching statistical significance, $t(91) = -1.925$, $p = .057$. Statistically significantly higher BUN values ($M = 23.42$, $SD = 5.66$) were obtained for the subsample with excessively high vitamin B12, compared to other individuals ($M = 18.60$, $SD = 6.91$), $t(89) = -2.30$, $p = .024$. It appears that high vitamin B12 and folate concentrations in the geriatric population are related to worse renal function.
DISCUSSION

The current investigation is a retrospective chart review study of geriatric community-residing sample. The study showed mild association between vitamin B12 and folate status and cognitive deficits.

One purpose of the study was to identify a cut-off level for vitamin B12 concentration below which cognitive deficits can be observed. Three cut-off levels derived from prior research were used to define vitamin B12 deficiency in this study, 200 pg/mL, 250 pg/mL, and 300 pg/mL. No difference in performance on cognitive tests was found between vitamin B12 deficient and non-deficient subjects in the current study, using these three cut-off levels. Vitamin B12 deficient and non-deficient groups were not different in terms of diagnosis or diagnosis severity. This conflicts with research findings of inferior performance on different tests of cognitive function in subjects with vitamin B12 deficiency (e.g., Bernard et al., 1998; Lewerin et al., 2005). This discrepancy in findings may be due to a high level of vitamin B12 supplementation (55.9%), and related elevation of vitamin B12 concentrations in the study sample. Vitamin supplementation represents an important confounding variable in a relationship between vitamin B12 concentration and cognitive performance. In view of high prevalence of vitamin supplementation, we might have missed subjects with tissue B12 deficiency by using the three traditional cut-off levels. Research suggests that plasma vitamin B12 may not be an accurate measure of true tissue deficiency. Since the majority of subjects in this sample had vitamin B12 concentrations in the clinically normal range, median split was used to elucidate relationship between B12 levels and cognitive functioning. The concentration of 529 pg/mL was used to differentiate subjects with high and low vitamin B12 levels. Median split analysis yielded a finding of significantly better delayed verbal recall, assessed by LMII total score and LMII
retention percentage in patients with B12 laboratory measures above 529 pg/mL. This difference was independent of diagnosis, diagnosis severity, age or education. The two groups differed only in terms of vitamin B12 supplementation, with individuals with higher B12 levels using supplements at a higher rate. This may speak in favor of beneficial effects of vitamin B12 supplementation on some aspects of cognitive functioning. Alternatively, vitamin supplementation may mask original differences between pre-supplementation vitamin concentrations and cognition. Due to the cross-sectional nature of the study, no causal assumption can be made with confidence. Regardless of the cause, median split analysis suggests that there is a higher cut-off level (above the traditionally used levels) for vitamin B12 concentrations at which differences in cognitive performance become pronounced.

Another goal of the study was to determine which cognitive tests are most sensitive to lower vitamin B12 concentrations. As mentioned earlier, individuals with below the median B12 concentration values have worse delayed verbal recall, measured by LMII total score and LMII retention percentage. Weak positive correlation between B12 concentrations and LMII retention percentage provides an additional support for sensitivity of this WMS subtest. This finding is consistent with the prior finding of relationship between low vitamin B12 and memory deficits, measured by the WMS (Elias et al., 2005; Goodwin et al., 1983), but conflicts with the majority of studies that failed to find association between low vitamin B12 and memory (Bernard et al., 1998; Hassing et al., 1999; Jelicic et al., 2001; La Rue et al., 1997; Nurk et al., 2005; Teunissen et al., 2003). This discrepancy is likely due to research design and subject characteristics differences.

Weak negative correlation was found between vitamin B12 and digit span total score. This test of attention and immediate memory was previously found to be unrelated to B12 levels
(Lindeman et al., 2000; Tucker et al., 2005). However, previous researchers used subcomponents of the digit span test (i.e., digit span forward or digit span backward), rather than the total score. Generally, composite test scores provide less clinical utility, compared to the more informative component test scores. Whether higher vitamin B12 level is truly related to lower attention, or this correlation is a result of statistical artifact of multiple comparisons and is not of clinical utility needs to be investigated in a larger study. Such limited findings on the relationship between vitamin B12 and neurocognitive functions may be due to using serum vitamin B12 to measure vitamin B12 status. Many researchers argued that serum B12 is not an accurate measure of vitamin B12 status and cannot identify vitamin deficient individuals (e.g., Holleland, et al., 1999; Lindeman et al., 2000; Pennypacker et al., 1992). Using vitamin B12 as an indicator of vitamin deficiency instead of more accurate indicators of tissue deficiency, such as Hcy and MMA levels is a limitation of the study. Unfortunately, current medical chart review revealed that Hcy and MMA are not routinely ordered by physicians. Studies also find that prevalence of elevated cobalamin and folate metabolites (Hcy and MMA) is higher than prevalence of low cobalamin and folate deficiencies within the same populations (e.g. Miller et al., 2003), suggesting that vitamin B12 and folate measures underestimate true deficiency. For example, Marengoni et al. (2004) found that sixty-four percent of hospitalized geriatric patients with normal serum vitamin B12 and folate concentrations had high Hcy levels. This may explain why within the same sample, Hcy levels correlated with a greater number of cognitive test scores, than vitamin B12 levels (e.g., Lewerin et al., 2005).

Another objective of the study was to assess diagnostic utility of serum vitamin B12 in differentiating between different types of dementia-related cognitive impairments. Comparisons of vitamin B12 concentrations among different diagnostic groups yielded an interesting finding
of similarity between groups of patients with AD and MCI. Among the five diagnostic categories (i.e., AD, VaD, mixed dementia, MCI, and other type of dementia), patients with AD and MCI had the lowest serum vitamin B12. The observed similarity in vitamin B12 concentrations, despite significant differences in severity of cognitive impairment between AD and MCI groups suggests a common physiological process underlying cognitive decline. MCI represents a transition stage between normal aging and AD. It's possible that decrease in vitamin B12 levels occurs during early stages of cognitive decline marked by MCI, and disease progression to AD does not lead to further decline in vitamin status. Prior research found that low level of vitamin B12 level was a precursor to the onset of AD. Individuals with low vitamin B12 levels had a doubled risk of developing AD over three years, compared to those with normal vitamin levels (Wang et al., 2001). Also, disease progression was not related to changes in vitamin B12. A 3-year longitudinal study found that levels of Hcy, vitamin B12 metabolite remained stable over time in patients with AD, and were not related to duration of symptoms (Clarke et al., 1998).

Individuals with AD and MCI both had significantly lower vitamin B12 values, compared to individuals who received diagnosis of ‘other’ type of dementia. Difficulty with interpreting this difference involves heterogeneity of the diagnoses included in the later category that was designed to include individuals with Parkinson disease, multiple sclerosis, frontal lobe dementia, and alcohol-induced dementia unrelated to vascular or Alzheimer disease pathology.

Also, compared to patients with vascular and mixed etiologies of dementia, individuals with AD and MCI had lower levels of B12. Although these differences were not statistically significant, they may be of clinical significance. Difference in vitamin levels, despite similar levels of diagnosis severity in patients with AD and VaD might signify etiological differences of these two disorders. A study by Bottilieri et al. (2001) on the other hand, found lower vitamin
B12 in individuals with VaD, compared to AD. Generally, hyperhomocysteinemia in AD is suggested to be a consequence of concomitant vascular dementia (e.g., Folin et al., 2005). Our findings suggest that a non-vascular etiology or a combination of vascular and non-vascular etiology of MCI and AD are responsible for relatively lower vitamin B12 values in these two groups.

Analysis of relationship between folate and cognitive function was limited by several factors. First, analysis included a combination of measurements of red blood cell folate and serum folate. Whereas serum folate is affected by recent food intake, red blood cell folate is considered a more stable marker of folate storage, as it reflects tissue storage of folate (Ortega et al., 1997). Second, the upper measurement limit for serum folate (24 ng/mL) used in our study placed considerable limitations on the kind of statistical analysis that could be performed. This upper measurement limit truncated the range of the continuous variable, resulting in underestimated means, and inability to perform correlational analysis. Third, due to a low prevalence (less than 1%) of folate deficiency in the sample, no comparison of folate deficient and non-deficient subjects could be performed. Such low prevalence of folate deficiency is considerably lower than the 11% to 30% prevalence reported in the community-dwelling elderly population (Clarke et al., 2003; Lewerin et al., 2005; Selhub et al., 1993). High folic acid supplementation (57.8%) in the sample and the effects of the grain fortification program likely account for such high laboratory folate measures, and low prevalence of folate deficiency. Finally, just as serum vitamin B12 is not an accurate measure of vitamin deficiency, serum and RBC folate do not always reflect true tissue deficiency.

Moderate positive correlation was found between red blood cell folate and LMI slope, indicating better ability for learning verbally presented information embedded in a context of a
story in individuals with higher folate levels. This is consistent with the finding of a positive correlation between folate and a measure of verbal learning and memory (Duthie et al., 2002). Research strongly supports a relationship between folate and information encoding and retrieval processes (e.g., Hassing et al., 1999; Lindeman et al., 2000; Teunissen et al., 2003).

An unexpected negative correlation between RBC folate and performance on spatial span backward subtest is challenging to explain. Spatial span backward is a measure of attention and ability to keep information in the working memory and perform mental manipulation. Generally, research suggests a positive relationship between folate and various tests of attention and information processing speed (e.g., Duthie et al., 2002; Elias et al., 2006; Lindeman et al., 2000). Measures used to assess the construct of attention and processing speed included digit symbol test (Duthrie et al.), symbol search (Elias et al.), digits forward and color trail making tests (Lindeman et al.). The only other known study that used digit span backward to understand a relationship between folate and attention, found no relationship between folate and performance on the test (Tucker et al., 2005). Whether our current finding is a result of statistical artifact of multiple comparisons and limited sample size, or is of clinical importance, needs to be determined by future research.

Median split analysis found that individuals with higher folate level perform better on the BNT and Trails B. The BNT is a test of confrontational naming and assesses the ability to retrieve words (Lezak et al., 2004). Trails B places demands on attention, and requires mental flexibility and effortful executive processing (Lezak et al.). Previously conducted exploratory factor analysis of this neuropsychological battery resulted in a five-factor solution, with the BNT and Trails B loading on the same factor, named cognitive flexibility. Both tests are executive tasks and rely on frontal lobe functioning. Association between folate and performance on tests
of frontal lobe functioning has been supported by research (e.g., Goodwin et al., 1983; La Rue et al., 1997). Analysis of the composition of the high folate and low folate groups concluded that folate status is related to the severity of diagnosis and folate intake, rather than type of dementia diagnosis, age and education. The nature of the relationship between folate and diagnosis severity is unclear. For example, greater cognitive decline could lead to decreased dietary intake resulting in lower folate concentrations. Alternatively, hyperhomocysteinemia and decreased folate may lead to cardiovascular and neurological changes leading to dementia.

In the present study, impaired renal function (based on elevated serum creatinine) was highly prevalent (19.6%) and considerably higher than reported in other studies (e.g., 5.4% for men and 6.5% in women; Miller et al., 2003). Positive relationships were found between high vitamin B12 and folate concentrations and lower renal function. Relationship between renal function and vitamin absorption is complex and poorly understood. Elevated serum creatinine was found to cause increased concentrations of vitamin B12 and folate metabolites, serum MMA and Hcy (Rasmussen, Vyberg, Pedersen, Brochner-Mortensen, 1990; Soria et al., 1990). Since vitamin B12 absorption is impaired in patients with renal failure (Obeid, Ruhlmann, Kirsch & Herrmann, 2005), higher than normal serum B12 concentrations are needed to ensure adequate vitamin level in the tissue. This finding of higher vitamin concentrations in the presence of lower renal function might be a result of a more intense vitamin supplementation among individuals with impaired renal function. No information about the specific vitamin daily dosage was collected in this study. It is likely that individuals with poor renal function identified by their physician were encouraged to take vitamins at higher doses and with greater consistency. Due to the laboratory set upper limit for serum folate it was not possible to assess the prevalence and effects of excessively high serum folate in this sample. Lucock (2004) raised a concern about the
effect of exceeding desired folate levels. Remarkably, information on what constitutes folate "overdose" is limited in the literature. The assumption that greater vitamin concentration is automatically better has not been confirmed scientifically. Clinical laboratory established the normal range for red blood cell folate as greater than 280 mg/mL and for serum folate as greater than 5.4 ng/mL. Pteroylmonoglutamate (PGA), a synthetic form of folate that is used in supplements and fortified products metabolises into methylfolate that is used by the body (Lucock). However, maximal methylfolate saturation occurs at doses of 400 µg PGA or less (a dose that far exceeds routinely prescribed folate concentrations), with the remaining unmetabolized PGA accumulating in the blood. Lucock warns of the potential health risks of long-term exposure to unmetabolized PGA.

The chief strength of the present study is the extent of neurobehavioral assessment. The cognitive function tests used in the present study assessed multiple cognitive domains. Despite certain limitations, the non-experimental medical chart review design of the study offers good external validity and clinical application. Multifactorial interaction between vitamin status, vitamin supplementation, diagnosis type and severity, and neurocognitive functioning creates a very complex picture that is usually observed in the clinical setting. In summary, our study adds further evidence to the hypothesis of a positive relationship between vitamin B12/folate status and cognitive functioning in several neurocognitive domains. In view of common vitamin supplementation among the elderly, impairments in neurocognitive performance are seen at higher vitamin concentration levels. This may be due to selective effectiveness of vitamin supplementation in improving functioning in other cognitive domains, or due to an overall improvement across multiple cognitive domains with impairments seen in the most profoundly impaired areas. Prospective longitudinal study is needed to test these hypotheses.
Results of the present study should be interpreted in view of several limitations. The non-experimental design and the use of convenience sample places certain limitations on interpretability and generalizability of the findings. Subject characteristics limit generalizability of the findings to the middle class, predominantly female, and predominantly Caucasian individuals. Majority of subjects attended physicians on a regular basis, and were taking dietary supplements. They were more likely to have had a better nutritional status, compared to individuals from a lower socioeconomic status. Participants in the present study were unusually well nourished. The fact that only several neuropsychological tests yielded significant relationships with vitamin status should be considered in light of limited sample size, as well as specific characteristics of the sample (i.e., high prevalence of supplement use). Reliability of vitamin supplementation data in this study depended on the accuracy of physician's recording of vitamin intake in the medical chart, as well as patient's intake of prescribed vitamins.

This cross-sectional neurocognitive data provide practical information for health-care providers. Our findings have potentially important implications for intervention strategies with respect to vitamin-related cognitive deficits: 1) There appears to be a higher cut-off level that is above the traditionally used levels for vitamin B12 and folate concentrations at which cognitive deficits become more pronounced; 2) Better cognitive functioning in individuals with higher vitamin concentrations suggests beneficial effects of vitamin supplementation in the geriatric population; 3) Some cognitive abilities, such as visuospatial memory, visuoconstructional skills, and language may be less related to vitamin concentrations, and supplement treatment may not be beneficial in preventing or reducing cognitive deficits in these areas; 4) Using serum MMA and Hcy is not a common practice among physicians. It's recommended that serum MMA be used as an indicator of vitamin B12 status in clinical practice. Hcy can be used as a less specific
indicator of vitamin B12, vitamin B6 and folate status. However, with folate fortification using Hcy would likely mask vitamin B12 and vitamin B6 deficiencies.
### Table 1

*Prevalence of Chronic Conditions in Different Diagnostic Groups*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>AD ( (n = 41) )</th>
<th>VaD ( (n = 20) )</th>
<th>MCI ( (n = 18) )</th>
<th>Other ( (n = 10) )</th>
<th>Mixed ( (n = 13) )</th>
<th>Total ( (n = 102) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of HTh</td>
<td>13 (32%)</td>
<td>5 (25%)</td>
<td>4 (22%)</td>
<td>1 (10%)</td>
<td>3 (23%)</td>
<td>26 (26%)</td>
</tr>
<tr>
<td>History of HHcy</td>
<td>23 (56%)</td>
<td>14 (70%)</td>
<td>9 (50%)</td>
<td>4 (40%)</td>
<td>12 (92%)</td>
<td>62 (61%)</td>
</tr>
<tr>
<td>History of HTN</td>
<td>28 (68%)</td>
<td>17 (85%)</td>
<td>11 (61%)</td>
<td>5 (50%)</td>
<td>12 (92%)</td>
<td>73 (72%)</td>
</tr>
</tbody>
</table>

*Note. HTh = Hypothyroidism; HHcy = Hypercholesterolemia; HTN = Hypertension; AD = Alzheimer Dementia; VaD = Vascular Dementia; MCI = Mild Cognitive Impairment*
Table 2

*Vitamin B12 Values in Difference Diagnostic Groups*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>AD (n = 41)</th>
<th>VaD (n = 20)</th>
<th>MCI (n = 18)</th>
<th>Other (n = 10)</th>
<th>Mixed (n = 13)</th>
<th>Total (n = 102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 value, M (SD)</td>
<td>500.6 (225)</td>
<td>690 (433)</td>
<td>504.5 (242.5)</td>
<td>956 (584.3)</td>
<td>776 (494.5)</td>
<td>619.3 (386)</td>
</tr>
</tbody>
</table>

*Note.* AD = Alzheimer Dementia; VaD = Vascular Dementia; MCI = Mild Cognitive Impairment
REFERENCES


population: Ethnic and sex differences in cobalamin and metabolite abnormalities. 


