VITAMIN B_{12} DEFICIENCY AND COGNITIVE DEFICIT AMONG OLDER ADULTS

by

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(Under the Direction of Lloyd Stephen Miller, Ph.D.)

ABSTRACT

Cognitive difficulties in later life can be both aversive and disruptive to older adults and bring about the loss of functional independence. One precursor to cognitive impairment in late life may be vitamin B_{12} deficiency, and associated elevations in the metabolites methylmalonic acid (MMA) and homocysteine. However, there has been little consensus on a cause-effect relationship between B_{12} deficiency and cognitive decrements. Furthermore, there is some controversy with respect to the efficacy of B_{12} replacement in reversing associated cognitive decrements. The current experiments address such questions in a longitudinal study of cognitive ability in B_{12}-deficient versus B_{12}-adequate older adults before and following vitamin B_{12} supplementation. Participants were classified as B_{12} deficient based upon elevated MMA levels. Those with elevated MMA levels underwent oral B_{12} replacement therapy (1000 μg pills) with measurement of serum vitamin levels, and measurement of performance on a battery of neuropsychological tests measuring memory, processing speed, reaction time, attention, verbal fluency and visual abstract reasoning being administered, at three time points over a period of twelve months. In the first experiment, participants with elevated MMA levels at baseline testing showed cognitive impairment relative to those with normal MMA levels in the domains of memory, verbal fluency and abstract reasoning. In the second experiment, following a twelve month trial of vitamin B_{12} supplementation, those responding metabolically to treatment demonstrated marked and significantly better improvements in memory and abstract reasoning relative to the placebo group. Findings suggest that reducing plasma MMA concentrations by administering vitamin B_{12} supplements may provide protection against cognitive decline as well as reverse potential cognitive deficits related to vitamin B_{12} deficiency in this and other elderly populations.

INDEX WORDS: cobalamin, homocysteine, methylmalonic acid, cognition, aging

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CHAPTER 1

INTRODUCTION

Cognitive difficulties in later life can be both aversive and disruptive to older adults and bring about loss of functional independence and increasing levels of emotional distress (Willis, Jay, Diehl & Marsiske, 1992). The presence of marked cognitive deficits in multiple domains in later life is often due to irreversible factors such as Alzheimer's disease, vascular disease, Parkinson's or other neurologic illness, chronic alcoholism, or combinations of these and other diseases. However, a small percentage of syndromes exhibiting cognitive deficits have been suggested as being caused by reversible factors. These conditions, commonly referred to by clinicians as "reversible dementias" are often caused by factors such as adverse reactions to medications, drug interactions, depression, heart and lung problems which deprive the brain of adequate amounts of oxygen, and chemical imbalances caused by poor nutrition. Vitamin B_{12} deficiency, a problem estimated to affect between five and fifteen percent of older adults in the United States (Stabler, Lindenbaum & Allen, 1997) has been suggested as being one such reversible dementia; however, there has been little consensus on a cause-effect relationship between B_{12} deficiency and cognitive decrements and there is furthermore some controversy with respect to the efficacy of B_{12} replacement in reversing associated cognitive decrements.

Past research has demonstrated that in persons with vitamin B_{12} deficiency hematologic, psychiatric and neurologic abnormalities, including cognitive deficits, can exist (Naurath, Joosten, Reizler, Stabler, Allen & Lindenbaum, 1995; Healton, Savage, Brust, Garrett &

Lindenbaum, 1991; Lindenbaum et al., 1988; van Asselt et al., 2001). Current research is now suggesting that such abnormalities may in actuality be a specific result of elevations in one or both of the metabolites associated with vitamin B_{12} deficiency, namely methylmalonic acid (MMA) and homocysteine (tHcy). Elevations in these metabolites are thought to exert toxic effects on the central nervous system at a cellular level, in turn reducing production of myelin and creating white matter lesions in the brain and spinal cord. In 1998, the Food and Drug Administration mandated that all foods be fortified with folate, a vitamin that helps regulate homocysteine levels. Thus, the role of MMA as potentially the most sensitive indicator and possible precursor to cognitive deficits associated with vitamin B_{12} deficiency has since become of special interest. It is not entirely clear the extent to which MMA elevations negatively affect cognition or, furthermore, the specific cognitive domains most affected by deviations in MMA, tHcy and serum vitamin B_{12}.

Vitamin B_{12} replacement therapy in clinically B_{12}-deficient persons has been shown to be both effective and ineffective in correcting associated cognitive deficits. Some case studies suggest cognitive improvement with B_{12} supplementation is possible (Evans, Edelsohn & Golden, 1983; Goebels & Soyka, 2000), while others do not (Barcikowska, Czyzewski, Pfeffer & Zawitkowska, 1994). There is limited imaging support for the usefulness of B_{12} replacement therapy in bringing about resolution of white matter changes and cognitive deficits associated with B_{12} deficiency (Chatterjee, Yapundich, Palmer, Marson & Mitchell, 1996), and several studies suggest supplementation improves neurologic symptoms, including cognition (Lindenbaum et al., 1988; Martin, Francis, Protetch & Huff, 1992; Eastley, Wilcock & Bucks, 2000; van Asselt et al., 2001). However, other studies have not shown B_{12} replacement therapy

as effective (DeJong et al., 2001; Kwok et al., 1998) and have refuted cognitive deficits associated with vitamin B_{12} deficiency as a reversible dementia syndrome.

Consequently, much remains unknown regarding the role of vitamin B_{12} in cognitive impairment in late life and the possibility for reversal of cognitive symptoms through B_{12} supplementation. No single study has yet prospectively examined the effects of B_{12} supplementation in a large community population of older adults and allowed substantial time for cognitive improvements, as measured using a variety of reliable and valid neuropsychological measures, to take place.

Therefore, the objectives of this dissertation are to examine relationships between vitamin B_{12} deficiency and cognition, as well as the effectiveness of B_{12} replacement therapy in B_{12}-deficient elders. We document the presence and degree of vitamin B_{12} deficiency and cognitive impairment in a large sample of older adults not drawn from a special population, employ well-validated neuropsychological measures to identify and measure the presence and progression of cognitive symptoms, and allow sufficient time for potentially slow neuronal and subsequent cognitive recovery to take place following B_{12} replacement in deficient older adults. Chapter three examines the association of selected indices of vitamin B_{12} deficiency, namely deficient serum B_{12}, elevated MMA and elevated tHcy, and various indices of cognitive functioning, including memory, attention, processing speed, reaction time, verbal fluency, and visual abstract reasoning. Special emphasis is placed on the role of elevated MMA as both a sensitive indicator of clinical B_{12} deficiency as well as a possible mechanism through which cognitive deficits emerge in persons with clinical B_{12} deficiency. In chapter four, associations between clinical B_{12} deficiency and cognitive deficit are examined, and changes in cognition are assessed following a twelve-month program of B_{12} replacement therapy in B_{12}-deficient elders.

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CHAPTER 2

LITERATURE REVIEW

Background and Significance:

Research into the causes and repercussions of functional decline in older adults is one of the most practical and growing literatures in gerontology and the field of cognitive aging. The population of America is, after-all, swiftly growing older. The elderly age group is the fastest growing segment of the population, with the percent of Americans over age 65 currently comprising about twelve percent of the national population (U.S. Bureau of the Census, 2003). This percentage is likely to steadily increase as progressively more people live longer lives, due to advances in medicine and technology. As Americans live longer lives, however, increasingly more persons will lose their ability to live cognitively intact and thus, functionally independent lives.

Research indicates that deficits in cognitive skills such as memory, organizational ability, judgment, and fluency are some of the most disruptive and alarming problems in the lives of aging individuals and their family members (Doraiswamy, Steffens, Pitchumoni & Tabrizi, 1998; Royall & Polk, 1998). Cross-sectional studies have demonstrated strong and consistent associations between impaired cognition and physical disability (Barberger-Gateau & Fabrigoule, 1997; Bassett & Folstein, 1991). Prospective studies have similarly found cognitive decline to be a strong predictor of physical and functional disability, even after controlling for demographic variables (Sauvaget, Yamada, Fujiwara, Sasaki & Mimori, 2002). In a study by Willis, Jay, Diehl & Marsiske (1992), impaired cognitive ability was found to contribute toward

a decrease in basic and instrumental activities of daily living. Functional losses in turn decreased self-efficacy beliefs and emotional adjustment within the older adult population. The presence of cognitive impairment in later life has been associated with depressive symptomatology (Ross, Arnsberger & Fox, 1998) and feelings of anxiety (Forsell, Palmer, & Fratiglioni, 2003). Research has also demonstrated that the caregivers of persons with dementia experience greater levels of mental and physical health problems (Baumgarten, Battista, Infante-Rivard, Hanley, Becker, & Gauthier, 1992) than those caring for persons without cognitive impairment. Recently, data from our own laboratory has demonstrated that caregivers with impairments in cognitive function themselves demonstrate higher levels of depression, anger and potentially harmful behaviors toward the recipients for whom they care (Lewis, Miller & FRILL, 2001).

From such research it is clear that deficits in cognition have direct negative effects on functional status and life satisfaction among older adults. Cognitive deficits can also negatively affect the families and caregivers of older adults. As the older population of America continues to grow, investigation into possible causes of cognitive impairment among older adults is an important and relevant area of research. This includes investigation into the potential influence of nutritional deficits on cognitive health. Research promoting treatment for cognitive impairment in late life may bring about more functional and enjoyable lives for older adults and their families.

Nutrition in older adults:

Nutritional factors play a major role in maintaining good health throughout the lifespan. As people age, adequate intakes of various vitamins and nutrients become increasingly vital in maintaining physiological and psychological strength and well-being. A number of vitamins are essential in providing for adequate physical functioning in later life. Vitamin D, for example,

helps prevent natural declines in bone density and muscle strength (Rosenberg & Miller, 1992). Intake of vitamin C and antioxidants promote visual acuity and prevent cataract formation and healthy doses of vitamin E and zinc contribute to proper immune functioning (Rosenberg & Miller, 1992).

One of the most important vitamins contributing toward overall health in later life is that of vitamin B_{12}. This very important member of the B vitamin family is one of the most commonly deficient vitamins within the older adult population. Research estimates that between three and 40 percent of older adults are clinically B_{12}-deficient (Baik & Russell, 1999), and many estimate that approximately 15 percent of adults age 65 and older are vitamin B_{12}-deficient (Chui et al., 2001; Stabler et al., 1997). Recent studies performed by researchers at the University of Georgia have indicated that 23% of rural dwelling older adults in elderly nutrition programs have vitamin B_{12} deficiency (Johnson et al., 2003). Moreover, these B_{12}-deficient older adults were found to have higher rates of mild cognitive impairment than their non-deficient peers. The high prevalence rate of B_{12} deficiency among older adults in both the state of Georgia and throughout the United States is cause for concern and intervention, as B_{12} deficiency can lead to marked physical and cognitive impairment. Vitamin B_{12} deficiency can, however, be easily ameliorated and, in some cases, symptoms may be reversed.

Cognitive decline in older adults:

Longitudinal studies have demonstrated that, in general, cognition declines with increasing age (Colsher & Wallace, 1991; Wilson, Beckett, Bennett, Albert & Evans, 1999). Studies have shown, however, that on an individual level, wide variability is seen, with some persons showing marked decreases in cognition over time and others showing no decreases at all or even slight improvement. Some factors that commonly influence cognitive performance

include educational attainment (Inouye, Albert, Mohs & Sun-Kolie, 1993), occupational level (Gold et al., 1995), depression (Rabbitt, Donlan, Watson, McInnes & Bent, 1995), declining physical health and sensory losses (Hultsch, Hammer & Small, 1993), as well as nutritional deficits. Indeed, older people with low intake levels of vitamins E, B_6 and, as discussed previously, vitamin B_{12}, have been shown to perform worse on various cognitive tasks. In some cases, older adults with nutritional deficiencies maintain cognitive decrements over time, particularly when not treated (LaRue et al., 1997).

Some forms of cognition are more likely to change over time than are other forms. There is evidence from the field of cognitive aging that cognitive ability in late life declines in a progressive manner (Smyer, Schaie & Kapp, 1996). Deficits have been shown to be initially exhibited in the arena of fluid intelligence, or skills that are biologically determined, independent of experience or learning (Cattell, 1963; Horn, 1982). Many of these skills are complex and require flexibility in thinking, inductive reasoning, and decision making ability. They are commonly measured by tests of reaction time, attention, memory, processing speed, verbal fluency, and abstract reasoning.

Reaction time refers to the speed of neural pathways and of the visual and auditory functions. With slower reaction times come greater delays in receiving and transmitting messages through the sense organs. Reaction time has been found to decrease with increasing age and health variables have been found to influence response speed in older adults (Houx & Jolles, 1993; Lezak, 1995).

Attention refers to the ability to sustain movement toward a goal (Fuster, 1997). It can be characterized by both selective processes, such as focused attention and divided attention, as well as processes including alertness and vigilance. Persons who experience attentional impairment

due to injury or brain lesion have been found to experience impaired alertness and a basic lack or weakness of drive (Zoccolotti et al., 2000). Additionally, such persons are unable to sustain concentration for prolonged periods of time, making task completion difficult (Fuster, 1997; Luria, 1966).

Memory is the process of retrieving or recalling information stored in the brain when needed. Three separate types of memory have been distinguished: sensory, primary, and secondary. Studies have demonstrated sensory memory as relatively stable across the life span (Lezak, 1995; Walsh & Thompson, 1978). Sensory memory is the first step in receiving information with sense organs before passing it on to primary memory, a temporary stage of holding and organizing information. For information to be permanently retained in secondary memory, or long-term memory, it must be sufficiently rehearsed and processed in primary memory. Research demonstrates that primary memory becomes vulnerable to aging when the memory task requires mental manipulation of the material (such as when reversing a string of digits) or when one is attempting to remember the information while engaging in another activity (Wingfield, Stine, Lahar & Aberdeen, 1988). Generally, it has been demonstrated that learning ability diminishes with aging, particularly when learning is measured by recall as opposed to recognition techniques (Lezak, 1995; Small, Stern, Tang & Mayeux, 1999).

Processing speed refers to the rate at which information is encoded, processed, and efficiently manipulated for use and/or storage in secondary memory. The decrease in processing-speed observed in older adults has been suggested to be a general mechanism underlying age-related differences on virtually all cognitive tasks, including various forms of memory as well as other abilities such as reasoning or spatial cognition (Salthouse, 1993). Information processing speed tends to decrease as adult age increases (Myerson, Hale, Wagstaff, Poon & Smith, 1990).

Fluency refers to the ability to actually produce effective performance on cognitive tasks. It involves the ability to organize items of thought, perception and action into goal directed thinking, speech, or behavior. It furthermore involves the ability to engage in productive activity in the absence of clearly defined parameters. Verbal fluency tasks demand the retrieval of words that meet certain criteria, for example, words beginning with a certain letter. It is generally held that this type of verbal fluency places demands on frontally mediated strategic search processes and executive functioning (Gleissner & Elger, 2001). Persons who lack cognitive fluency are unable to generate novel responses or vary their words and actions in daily life. Research has found that fluency steadily declines with increasing age (Ylikoski et al., 1999).

Abstract reasoning is the ability to generalize from specific facts to concepts and to use these concepts to correctly understand and manipulate information. It is considered to be a higher cognitive function, or one that involves complex mental operations, and it is relatively sensitive to brain injury, including lesions in the brain, regardless of site (Lezak, 1995)

Performance in all of these areas has been found to generally decrease over time. However, change in these areas in later life is highly specific to the individual and the various environmental and genetic factors that influence that individual (Wilson et al, 2002). This study aims to further clarify the role that one such variable, vitamin B_{12} deficiency, may have on these areas of cognitive functioning in later life.

Health concerns associated with vitamin B_{12} deficiency:

Vitamin B_{12}, is a micronutrient that has been found to contribute toward efficient bodily functioning and health. It is essential for normal metabolism in every cell of the body, particularly in the stomach and intestines, bone marrow and the central nervous system (CNS). It assists in the production and maintenance of myelin sheaths and plays a major role in the

function of the nervous system. B_{12} is also needed for two enzymatic reactions in humans that regulate the amount of certain biochemicals, methylmalonic acid (MMA) and homocysteine (tHcy), circulating in the blood. The first enzymatic reaction converts methylmalonyl-coenzyme A (CoA) to succinyl-CoA using vitamin B_{12} as a cofactor. A deficiency in vitamin B_{12} leads to an increase in serum methylmalonyl-CoA and its metabolic product, methylmalonic acid. Secondly, the presence of B_{12} is a necessary component in the synthesis of methionine from homocysteine. Folate is the methyl donor in this metabolization, or remethylation, reaction. Methionine is an essential amino acid of the body that helps generate DNA and RNA within cells and myelination throughout the CNS. Breakdowns in the synthesis of methionine, due to either a B_{12} deficiency or a folate deficiency, lead to the accumulation and elevation of tHcy. Again, either B_{12} or folate deficiency, and to a lesser degree vitamin B_6 deficiency, lead to the elevation of tHcy (Stabler et al., 1988). Thus, serum MMA concentration is the currently preferred indicator of vitamin B_{12} status because, although it too can be elevated due to factors other than B_{12} deficiency such as in cases of chronic renal failure, it is a more specific indicator of B_{12} deficiency than elevations in tHcy (Baik & Russell, 1999; Morris, Jacques, Rosenberg & Selhub, 2002; Savage, Lindenbaum, Stabler & Allen, 1994).

The presence of both folate and vitamin B_{12} deficiency have been linked to hematologic problems in humans, such as megaloblastic anemia. Megaloblastic anemia is a condition in which an inadequate number of red blood cells are produced. Additionally, existing red blood cells demonstrate nuclear dysmaturity, a condition in which the nucleus of the cell appears immature relative to its cytoplasm due to impaired DNA synthesis. White blood cells, consequently, are enlarged and many cells die in the bone marrow. Physical symptoms associated with megaloblastic anemia include weak muscles, weight loss, difficulty walking, lack

of energy and increased fatigue, gastrointestinal distress including diarrhea and nausea, tachycardia, irritability, and the presence of a smooth and tender tongue. Because the metabolism of folate and vitamin B_{12} is closely connected, a deficiency in either vitamin can cause this morphologically indistinguishable type of anemia and virtually no hematologic findings can distinguish between the two conditions. Hematologically, vitamin B_{12} deficiency is identical to folate deficiency. Vitamin B_{12} deficiency, however, has been suggested as a precursor to neurologic and cognitive problems and has been suggested as being a potentially reversible form of dementia. Past studies have demonstrated that when B_{12} deficiency is recognized and treated through supplementation in a timely manner, improvements in neurologic symptoms, including cognition, can result (Lindenbaum et al., 1988; Martin et al., 1992). Failure to recognize B_{12} deficiency, even in the presence of concomitant folate deficiency, may result in permanent neuropsychiatric damage. Thus, it is important to distinguish between B_{12} deficiency and a folate deficiency because administering folate to a vitamin B_{12}-deficient person will normalize folate levels, in turn correcting hematologic symptoms associated with both folate and B_{12} deficiency. The underlying B_{12} deficiency, however, may remain undetected and can progress to neurological disorders if left untreated. The megaloblastic anemia will be corrected with folic acid supplements, but the vitamin B_{12} deficiency will become masked and neurological degeneration will persist (Stabler et al., 1997). The distinction between folate and vitamin B_{12} deficiency is particularly important today, as in 1998 the FDA mandated that a variety of foods, such as flour, pasta, and rice be fortified with folate in order to prevent the development of hematologic impairments. While the increase in dietary folate is helping to prevent anemia and other hematologic impairments in older adults, the presence of adequate folate does not ensure, nor even suggest, the presence of sufficient vitamin B_{12}. It is imperative that the presence and

influence of vitamin B_{12} deficiency in older adults be adequately measured and understood in order to prevent and/or treat the associated neurologic and cognitive deficits.

Interestingly, an inverse relationship has often been found between the severity of the neuropsychiatric effects of B_{12} deficiency and severity of the hematologic effects, with only about one third of patients with megaloblastic anemia developing neurological deficits (Savage et al., 1994). Such a finding further underscores the importance of sensitive testing for the presence of B_{12} deficit, in addition to folate deficiency, as neuropsychiatric symptoms associated with B_{12} deficiency may not be discovered through standard folate and hematological assessment measures.

The precise nature in which deficient B_{12} exerts its influence on bodily systems remains somewhat unclear and will be discussed further in this paper. Many have proposed that neuropsychiatric abnormalities arise in conjunction to subacute combined degeneration of the spinal cord (SACD). This demyelinating disorder of the CNS is brought about by methylation processes in B_{12}-deficient patients. Bottiglieri (1996) reported the most common neurological symptom associated with B_{12} deficiency as being peripheral neuropathy and that organic mental change, including the presence of dementia and/or cognitive impairment, was also associated with deficient levels of vitamin B_{12}. Other neuropsychiatric signs and symptoms found to be associated with B_{12} deficiency include poor visual and auditory processing, hallucinations and/or changes in personality, and abnormalities of joint-position sense, cutaneous touch and pain sensation, reflexes, and gait (Baik & Russell, 1999; Dharmarajan & Norkus, 2001; Lindenbaum et al., 1988; Stabler, 2001).

The development of vitamin B_{12} deficiency:

Vitamin B_{12} is found in a variety of common foods. Eggs, meat, poultry, milk, shellfish and fortified breakfast cereals are all rich in vitamin B_{12}. The recommended B_{12} dietary allowance (RDA), or the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all healthy adults is 2.4 micrograms for both men and women who are neither pregnant nor nursing (Institute of Medicine, 1998). Daily multivitamins often supply the body with several times this amount of B_{12} ; however, vitamin B_{12} has a very low potential for toxicity and, after being stored by the body in the liver and kidneys, excess vitamin B_{12} is passed from the body through the urine and feces. Yet, even with a wide variety of available B_{12} rich foods and high microgram supplements, many older adults are B_{12}-deficient.

In order to understand the reasons accounting for vitamin B_{12} deficiency, it is important to understand how B_{12} is absorbed by the body. Vitamin B_{12} is bound to the protein in food. Hydrochloric acid (HCL), the acid found in stomach secretions, releases B_{12} from its peptide bonds during the process of digestion. Once released, B_{12} combines with a different protein found in gastric juices, called intrinsic factor, which facilitates the vitamin's absorption into the bloodstream. A large portion of the B_{12} is absorbed into the bloodstream through the ileum, a portion of the small intestine. It is then transferred throughout the body to facilitate healthy functioning and maintenance in the nervous system, growth of body tissues, and maturation of red blood cells. Breakdowns in the normal absorption and distribution of B_{12} can occur at any point in this absorption process. Research has shown that serum vitamin B_{12} concentrations decrease with age among healthy older populations (Lindenbaum et al., 1994).

Breakdowns most commonly happen in the body's ability to absorb vitamin B_{12} in the stomach. Dietary deficiency of B_{12} is rare due to the large availability of B_{12} rich foods; however,

vegetarians, as well as older adults with dietary restrictions, are at a higher risk for B_{12} deficiency and are often counseled by doctors to take extra measures to ensure sufficient consumption of B_{12} supplements and enriched foods. B_{12} deficiency related to malabsorption can occur for a variety of reasons at many stages of the absorption process. One of the most common causes of malabsorption is the body's inability to make sufficient levels of HCL needed to separate vitamin B_{12} from its attached proteins. Often, the presence of certain diseases or the use of various medications can reduce HCL production. Atrophic gastritis, a condition found to affect between nine and thirty percent of older adults (Baik & Russell, 1998), involves the inability to secrete sufficient HCL to kill bacteria found in both the stomach and small intestine. As a result, in older adults suffering from atrophic gastritis, B_{12} is limited due to both impaired release of B_{12} from food proteins, as well as the overgrowth of gastrointestinal bacteria sapping existing B_{12} for the bacteria's own use. Whereas food bound vitamin B_{12} is diminished in this disorder, crystalline B_{12} (or that found in vitamin pills) is unaffected.

Another common problem in the B_{12} absorption process is due to a lack of intrinsic factor in gastric secretions, a condition called pernicious anemia. As mentioned previously, intrinsic factor is necessary in allowing for adequate absorption of B_{12} from the ileum into the bloodstream. In persons with pernicious anemia, less than 1% of the B_{12} in food is absorbed. Rather, vitamin B_{12} is excreted in stool, in turn leading to the development of B_{12} deficiency. It is estimated that approximately two percent of physically healthy older adults are affected by pernicious anemia, with women and people of Scandinavian or Northern European descent being at slightly higher risk (Baik & Russell, 1999). Additionally, a variety of disorders can include poor absorption of B_{12} as a related complication. Such disorders include pancreatic insufficiency,

terminal ileal disease, lymphoma, radiation enteritis, intestinal tuberculosis, and celiac disease (Baik & Russell, 1999).

In addition to malabsorption as a cause of vitamin B_{12} deficiency, less common antecedents to B_{12} deficiency include the inhalation of the anesthetic nitrous oxide. Nitrous oxide brings about inactivation of B_{12} and, in turn, the clinical features of B_{12} deficiency. Nitrous oxide is commonly used in surgery; thus, in an older person, existing B_{12} deficiency is often ruled out before using this drug while operating. Vitamin B_{12} deficiency is often considered present in cases of post-operative neuropathology (Baik & Russell, 1999).

Mechanisms through which vitamin B_{12} exerts its effects:

While a variety of researchers have demonstrated the presence of a relationship between deficient B_{12} levels and poor cognitive functioning (e.g., Lindenbaum et al., 1988; Martin et al., 1992), the precise mechanism driving this relationship remains unclear. Also unclear is the degree of positive cognitive change that can be brought about through B_{12} supplementation in cases of cognitive impairment and borderline cognitive functioning due to B_{12} deficiency. As previously mentioned, deficient levels of B_{12} can result in the bodily build up of the biochemicals MMA and tHcy, the impaired production of the amino acid methionine, and the production of immature red blood cells. Some have theorized that such developments are intricately linked to neurological impairment at a cellular level, as well as cognitive problems including dementia, vascular disease, and Alzheimer's disease (Bottiglieri, 1996; Lindenbaum et al., 1988).

One of the leading theories regarding how B_{12} deficiency exerts its influence on cognition is the hypomethylation hypothesis. This theory proposes that a lack of B_{12} and folate contribute to a lack of methionine, which in turn limits necessary myelin, neurotransmitters, and membrane phospholipids crucial for function of the nervous system. Abnormalities in central nervous

system myelin have been supported in a number of studies. Animal studies have demonstrated that rats undergoing total gastrectomy, or removal of the stomach which in turn depletes intrinsic factor and B_{12} production, show insults to the integrity of myelin in the CNS (Scalabrino et al., 1990). Myelination abnormalities have been shown to include the presence of subacute combined degeneration (SACD), or uneven and irregular demyelination in the spinal cord, and fully developed white matter lesions throughout the CNS. Animal studies in species other than rats give further evidence for the presence of SACD and associated brain and spinal cord lesions being associated with defects in methylation. Lesions in the brain, optic nerves, and peripheral nerves have been observed in non-human primates (Scott et al., 1981), and fruit bats (Metz & van der Westhuyzen, 1992).

Genetic studies support such animal research. Hyland et al., 1988, demonstrated that children born with congenital defects of both vitamin B_{12} and folate metabolism had severe and progressive demyelination and disease of the CNS. Remyelination throughout the CNS was accomplished in these children through supplementation of S-adenosylmethionine (SAM), the product of methionine synthesis. Furthermore, using magnetic resonance imaging, Chatterjee et al., 1996, presented a case report in which a man with cognitive impairment associated with B_{12} deficiency and confluent white matter abnormalities showed marked cognitive improvement with B_{12} replacement therapy. Imaging abnormalities partially resolved with B_{12} replacement therapy. It is clear from such studies that vitamin B_{12} deficiency has a significant effect on the integrity of myelin and white matter.

An alternative theory to the hypomethylation hypothesis, called the homocysteine toxicity hypothesis describes a process in which homocysteine accumulation in the blood and other tissues becomes neurotoxic to the body's cells. This in turn has neurological consequences.

As mentioned, tHcy is an amino acid that is formed during the metabolism of methionine. It is metabolized by one of two pathways: remethylation and transsulfuration. Vitamin B_{12} is an essential cofactor in the remethylation cycle. If vitamin B_{12} status is inadequate, tHcy will not be metabolized to methionine and tHcy concentrations will increase. The mechanism of the neurotoxic effect is likely the result of homocysteine's metabolism to S-adenosylhomocysteine (SAH), causing inhibition of methylation reactions in the CNS. Evidence for such a mechanism has been supported in animal studies. Schatz, 1981, found, for example, that in rats, tHcy elevation in the brain led to increases in SAH and concomitant inhibition of monoamine neurotransmitter metabolism, protein and phospholipid methylation.

The build up of homocysteine can also lead to cell death through excitotoxic effects on the neurons. Homocysteine is oxidized to homocysteic acid (HC-SO3) which, in turn, is generated in brain tissue. HC-SO3 is an excitatory agonist at NMDA receptors in the brain. NMDA receptors are a subgroup of excitatory glutaminergic receptors involved in synaptic transmission and which are important in long-term potentiation. Activation of NMDA receptors results in a rise of intracellular calcium, the consequent release of cellular proteases, and eventual cell-death. HC-SO3 causes more potent activation of the pre- and post-NMDA receptors than glutamate. Thus, in patients with B_{12} deficiency, accumulated tHcy metabolizes to HC-SO3, becomes neurotoxic via NMDA receptor activation and may be ultimately responsible for neurological and cognitive abnormalities.

Buildup of the metabolite MMA has been found to impact neurological functioning through similar processes as those used by tHcy, namely excitotoxic and neurotoxic activity at a cellular level in the CNS. Animal research has demonstrated that the effects elicited by MMA may lead to excessive glutamate concentrations at the synaptic cleft, thus producing excitotoxic

cellular activity and eventual cell death (Brusque et al., 2001). Animal studies have demonstrated that the buildup of MMA can provoke excitotoxic neuronal damage in vitro using neurons cultured from both chick embryos and rats (Mello et al., 1996). Further support comes from patients diagnosed with methylmalonic acidemia. Methylmalonic acidemia is an inherited metabolic disorder usually diagnosed in infancy that causes the accumulation of MMA in the body. The buildup of MMA in these patients is brought about by a defect in the metabolic conversion of methymalonyl-coenzyme A to succinyl-CoA and a secondary accumulation of MMA. Patients with this disorder have been shown to experience severe metabolic and neurologic disturbances. Features of the disorder include chronic disability, seizures, and developmental delay. Imaging techniques including MRI and CT have shown a cerebral picture of hypomyelination, cerebral atrophy, and edema of white matter. Interestingly, when such patients are diagnosed early and treated effectively by keeping MMA values near normal levels, they may be neurologically normal. Such findings support the philosophy that MMA accumulation in this disorder is neurotoxic and that by controlling MMA, some of the most serious neurological deficits may be controlled (Brusque et al., 2001).

In summary, research has demonstrated that deficient vitamin B_{12} contributes to hematologic and neurologic difficulties. Even in cases in which serum B_{12} levels are in the low to low-normal range, but there exist elevated metabolite levels, cellular vitamin B_{12} deficiency may be present and having excitotoxic or neurotoxic affects on the CNS. The precise relationships between tHcy, MMA, vitamin B_{12}, and their unique contributions to physical and cognitive functioning remain unclear. It is increasingly important to understand such relationships, however, as folate is now supplemented in many foods, in turn contributing to the normalization of tHcy levels due to sufficient folate in B_{12}-deficient patients. Although tHcy and

serum B_{12} levels may be generally normal, it remains possible that vitamin B_{12} deficiency at a cellular level, as indicated by elevated MMA levels which do not respond to folate supplementation, is contributing to neurological and cognitive deficits.

Vitamin B_{12} deficiency and hematologic impairment:

Although the mechanism driving neurologic and hematologic impairment due to B_{12} deficiency remains somewhat controversial, a growing body of literature supports the existence of important relationships between B_{12}, MMA, tHcy, and overall bodily and cognitive health. It is currently well known that B_{12} supplementation can effectively bring about positive changes in hematologic abnormalities and the metabolic functioning of the CNS. Supplementation of B_{12} in B_{12}-deficient patients treats the presence of megaloblastic anemia, normalizes elevated mean white blood cell volumes, folate levels, and can significantly reduce levels of MMA and tHcy (Allen, Stabler, Savage & Lindenbaum, 1990; Lindenbaum et al., 1988; Naurath et al., 1995).

In fact, research has demonstrated that even among older adult research participants with normal levels of serum vitamin B_{12}, supplementation can lower and in turn normalize elevated levels of MMA and tHcy (Naurath et al., 1995). This lowering of metabolic levels following B_{12} supplementation supports the notion that metabolic evidence of vitamin deficiency is common even among persons with normal vitamin serum levels. Intracellular vitamin deficiency may be subclinical, apparent only through the presence of elevated metabolites (MMA and tHcy). Bjorkegren & Svaardsudd, 1999, similarly found that B_{12} supplementation has positive metabolic effects, even among persons within the conventional laboratory ranges of 'normal' serum vitamin B_{12} level. In a population based intervention study, these researchers found that oral or intramuscular B_{12} supplementation normalized elevated MMA values; furthermore, treatment combining B_{12} and folic acid supplementations normalized elevated tHcy levels. In a similar

study, Fenech, Dreosti & Rinaldi, 1997, implemented a diet supplemented with B_{12} among older adults and again found that a significant positive alteration in folate status, tHcy level, and the integrity of the cell micronucleus resulted. In sum, studies regarding the relationship between vitamin B_{12} and hematologic factors have demonstrated that metabolic signs of clinical B_{12} deficiency, namely elevated MMA and tHcy, can exist even in the presence of normal laboratory serum B_{12} levels, and that elevated metabolites and other hematologic abnormalities can be normalized through B_{12} supplementation.

Vitamin B_{12} deficiency, neurologic and cognitive impairment:

While many studies give clear evidence of metabolic improvements with B_{12} supplementation, the clinical relevance of B_{12} supplementation in the realm of neurocognitive function remains unclear. Many researchers have suggested that B_{12} supplementation in B_{12}-deficient persons can improve neurological and cognitive function, as well as dementia. A body of literature also exists, however, refuting B_{12} supplementation as a valid treatment for cognitive symptomology.

Support for the usefulness of B_{12} supplementation comes from both studies of psychological well-being and studies of neurologic and cognitive function. Researchers have found that a variety of psychiatric symptoms hypothesized as being brought about by deficient B_{12}, including agitation, paranoia, irritability and disorientation, are partially or completely reversed with B_{12} supplementation (Healton, Savage, Brust, Garett & Lindenbau, 1991; Evans, Edelsohn & Golden, 1983). Case reports exist in which persons with B_{12} deficiency have presented with psychiatric symptoms but without hematological signs of deficient B_{12} levels (Binder, 1983). In such cases, careful case analysis and treatment with B_{12} supplementation have brought about reversal of psychiatric symptoms.

The role of vitamin B_{12} in cognitive and neurological health is becoming an increasingly exciting topic of investigation. Organic mental change, including dementia and/or cognitive impairment, has been linked to B_{12} deficiency in both healthy older adults (Bohnen, Jolles & Degenaar, 1992; Duthie et al., 2001; Goodwin, Goodwin & Garry, 1983; La Rue et al., 1997; Riggs, Spiro, Tucker & Rush, 1996) and impaired older adults (Clarke et al., 1998; Kristensen et al., 1993). In 1983, Goodwin, Goodwin & Garry demonstrated that non-demented patients with low B_{12} levels scored relatively poor compared to control subjects on verbal memory tests and on the Halstead Reitan Categories Test, a test of cognitive flexibility and fluency that requires one to organize and conceptualize abstract visual information. While Goodwin and colleagues did not find linear relationships between B_{12} status and cognitive function, they did demonstrate that participants with the lowest levels of B_{12} scored significantly worse than persons with the highest levels of serum B_{12} on neuropsychological measures. More recently, other researchers have used similar statistical techniques to demonstrate associations between B_{12} deficiency and cognitive functioning. In 1992, Bohnen, Jolles, and Degenaar studied the effects of vitamin concentrations on older adults' cognitive function by classifying participants as having low or normal vitamin B_{12} levels. They then determined whether participants with low vitamin concentrations performed worse on cognitive tasks than those with normal vitamin levels. Findings showed that those with low vitamin B_{12} levels performed worse on a test of complex information-processing speed than those with normal B_{12} levels. Recently, Jelicic, Jonker, and Deeg (2001) again demonstrated, using multivariate analyses, that research participants with deficient levels of B_{12} (<210 pmol/L) exhibited reduced information processing speed relative to participants with normal vitamin B_{12} levels. Wahlin et al. (2001) gave further support for a relationship between deficient B_{12} and impairment on tasks which require fast and accurate processing of novel

information by finding that persons with low levels of B_{12} (<200 pmol/l) demonstrated impaired performance relative to controls on block design and letter fluency subtests, or tasks theorized as utilizing fluid intelligence. Vitamin B_{12}-deficient older adults demonstrated generally intact performance on tests utilizing preexisting knowledge structures, or tasks tapping into crystallized intelligence. Like previous researchers, Wahlin and colleagues suggested that the effects of vitamin status on cognitive performance may be seen most easily below certain thresholds.

Steadily, some linear relationships between B_{12} deficiency and impaired cognition are being discovered. Riggs et al. (1986) found linear relationships between vitamin B_{12}, elevated tHcy, and performance on a spatial copying task; however, they failed to find significant relationships between vitamin status and a number of other cognitive tasks. In 1997, LaRue et al. demonstrated the presence of correlations, though modest, between cognitive performance and vitamin status. And, recently, Duthie et al. (2001) reported that among a 78 year-old cohort in Scotland, who were first tested as part of the Scottish Mental Surveys of 1932, the presence of deficient vitamin B_{12} was positively related to impairment on the Mini Mental State Exam, a screen of overall cognitive functioning. Inverse relationships were found present between elevated tHcy and performance on neuropsychological tasks of non-verbal spatial reasoning, speed of information processing, and visuospatial organization. Furthermore, after adjusting for childhood IQ levels, age, and education, tHcy levels remained associated with neuropsychological tasks in regression analyses, accounting for an additional seven to eight percent of the variance on tests of cognitive functioning.

Increasingly, focus is turning to relationships between elevated metabolites, as opposed to deficient serum B_{12} levels, and cognitive functioning. Recently, Miller and colleagues (2003) looked specifically at associations between elevated tHcy levels and cognitive functioning. Using

multiple regression analyses in their study among elderly Latinos, they found that tHcy was inversely correlated with seven tasks of cognitive function including global functioning, memory, attention, and abstract reasoning tasks. They found that tHcy explained from two to five percent of the variance in cognitive function scores within the sample. The inclusion of demographic variables, however, such as age, sex and acculturation, significantly attenuated the relations between tHcy and cognitive function. Nonetheless, tHcy was found to be a modest independent predictor of cognitive function in community-dwelling elderly Latinos. Similar investigations into the association between elevations of the metabolite MMA and cognitive functioning are to date lacking in the existing literature. Such studies are increasingly important given that adequate folate levels may be contributing to adequate tHcy levels, but leaving MMA levels elevated, as indicative of underlying clinical vitamin B_{12} deficiency in older adults. It has been suggested that Miller et al.'s study would have been strengthened by measurement of MMA, so as to more precisely define the role of vitamin B_{12} deficiency in impaired cognition, now that folate deficiency has been largely removed as a variable (Stabler, 2003).

Among cognitively impaired older adults, researchers have found that some patients with Alzheimer's disease have either low serum vitamin B_{12} or low cerebrospinal fluid (CSF) vitamin B_{12} (Ikeda et al., 1990), as well as elevated levels of homocysteine (Clarke et al., 1998). Additionally, mean MMA levels have been found to be higher in groups of Alzheimer patients compared to groups of other dementia patients or psychiatric patients (Kristensen et al., 1993).

Taken together, cross sectional research in healthy and cognitively impaired older adults has demonstrated non-causal relationships between vitamin B_{12} deficiency and cognitive impairment, ranging from the presence of dementia (Clarke et al., 1988; Kristensen et al, 1993) to impaired performance on tests of global measures of intelligence (Duthie et al, 2001) to poor

cognitive function in specific areas of cognitive function including processing speed (Bohnen et al., 1992; Jelicic, Jonker & Deeg, 2001), constructional praxis (Riggs et al., 1996; Duthie et al., 2002), attention (Bohnen et al., 1992), and verbal fluency (Wahlin et al., 2001). Continued research is warranted to understand associations between elevated metabolites, specifically MMA elevations, and impaired cognition and, furthermore, the mechanism through which vitamin B_{12} deficiency exerts its effects on cognition.

Cognitive changes associated with vitamin B_{12} supplementation:

Can vitamin B_{12} supplementation alleviate cognitive losses found to be associated with B_{12} deficiency? Some case examples exist in the literature detailing neuropsychiatric recovery in persons with B_{12} deficiency (Goebels & Soyka, 2000; Chatterjee et al., 1996). Through such case studies, substantial evidence has been presented for the crucial involvement of vitamin B_{12} in a variety of pathophysiological conditions affecting the CNS, ranging from myelination to transmitter function. However, the case examples failed to delineate a causal relationship between B_{12} deficiency and dementia and, furthermore, did not distinguish the effects of B_{12} supplementation from the effects of comedication, supporting therapeutic measures, and retest improvement. Moreover, additional case examples exist in which B_{12}-deficient patients have not recovered from dementia through B_{12} supplementation (eg., Marino, Grassi, Macciardi & Scarone, 1994).

In an attempt to clarify the use of B_{12} supplementation in cases of cognitive loss, Lindenbaum et al. (1988) retrospectively studied 141 patients admitted to a large medical center with low B_{12} levels and a variety of cognitive and psychiatric abnormalities. Lindenbaum found that the most common symptoms associated with B_{12} deficiency were those of parasthesia, ataxia, memory loss, and limb weakness. All patients enrolled in the study benefited from B_{12}

therapy. Improvements included positive changes in metabolic findings (decreases in MMA and tHcy levels) as well as improvements in neurological abnormalities. Lindenbaum pushed his research one step further in a prospective follow-up study with 40 patients in which present neurologic symptoms appeared to be due to B_{12} deficiency and who demonstrated no signs of anemia. Researchers demonstrated that 100% of the patients showed improvement in some of their neurologic symptoms with B_{12} therapy. For example, of 13 patients with memory loss, eight were reported to have shown complete recovery and three to have shown partial recovery with B_{12} supplementation. Of 15 patients with abnormal gait, seven made a complete recovery and five demonstrated improvement, and of 30 patients with parasthesia, 21 showed a complete recovery with the remainder showing at least partial recovery.

Even as such research gives evidence of positive clinical changes as related to B_{12} supplementation, such studies have been criticized for containing referral biases, lack of vigorous diagnostic criteria, lack of objective neuropsychological data, and reliance on general clinical impressions to gauge response to treatment. To examine cognitive changes, existing studies have primarily made use of short mental status screens that may not be sensitive to change over time or adequately measure abstract reasoning and constructional ability. In response to such methodological inadequacies in the literature, Martin et al., 1992, conducted an eight-month open trial of B_{12} therapy among 18 patients suffering from cognitive impairment and found to have low serum B_{12} levels. Using the Mattis Dementia Rating Scale (DRS), a well validated and reliable neuropsychological measure which measures cognitive change over time, Martin found that 11 of 18 patients improved in mental status over the eight month treatment period, with the greatest gains in memory, construction, initiation and conceptualization. All responders were found to be in only the mild range of cognitive impairment at baseline measure. Also, a short

duration of pre-treatment mental symptoms (i.e., cases in which symptoms persisted for less than one year) was found to be related to cognitive improvement with B_{12} therapy. Thus, Martin used sound methodology and valid neuropsychological measures to demonstrate that, at least in some cases, cognitive remediation through B_{12} supplementation appears possible. His findings supported the work of several past researchers in suggesting that cognitive improvement seems to be possible only in early stages of impairment, before structural changes occur without the possibility of neuronal repair (Freemon & Rudd, 1982; Larson, Reifler, Featherstone & English, 1984).

In 2000, Eastley, Wilcock and Bucks used neuropsychological test data to examine the effects of B_{12} treatment on cognitive function and disease progression in patients presenting with dementia or cognitive impairment. In their retrospective study, 125 patients were found to have low serum B_{12}. Sixty-six of these patients presented with dementia and showed no significant improvement, and no less deterioration, in their neuropsychological function compared to matched controls. However, among 22 patients presenting with cognitive impairment, as opposed to dementia, a treatment effect was demonstrated The MCI patients improved significantly compared to matched patients on tests of verbal fluency, a cognitive skill theorized as involving frontal cortical activity. Such results were interpreted as suggesting that while vitamin B_{12} treatment improved B_{12}-deficient patients' cognitive function in the realms of language and frontal lobe functioning, treatment did not significantly improve the cognitive status of persons with dementia.

Recently, an interesting treatment study showed that vitamin B_{12} replacement improved cognitive performance and abnormalities on electroencephalogram in vitamin B_{12}-deficient seniors (van Asselt et al., 2001). Following B_{12} supplementation in a sample of 16 community

dwelling older adults, plasma B_{12} concentrations increased and MMA and tHcy levels decreased. Participants' performance on tasks of verbal learning, verbal fluency, and similarities improved and quantitative EEG showed more fast activity and less slow activity. This finding adds to previous research which has suggested improvements in some mental status scores can occur in B_{12}-deficient seniors with a short duration of pre-treatment mental symptoms (Martin et al., 1992), or in older adults with predominantly pre-treatment memory problems as opposed to more severe forms of cognitive impairment or dementia (Eastley et al., 2000).

Indeed, vitamin B_{12} replacement therapy has not been proven effective in improving mental symptoms in persons with long standing dementia (Carmel et al., 1995). Additionally, in contrast to previous findings, some research has demonstrated B_{12} supplementation therapy as also being ineffective in bringing about cognitive enhancement among community-dwelling adults with varying levels of B_{12} deficiency. In a randomized trial of the effect of supplementation on the cognitive function of older people with subnormal serum vitamin B_{12} levels (<120 pmol/l), Kwok and colleagues (1998) found that participants supplemented with intramuscular B_{12} for a period of three to six months improved in performance IQ, but the amount of improvement was not significantly more than that of control participants. Based on their findings, the authors noted that their hypothesis that vitamin B_{12} deficiency invariably causes cognitive impairment in older adults was unsupported. In a study in 2001, DeJong and colleagues again found no relationship between B_{12} level and neuropsychological functioning in a group of 217 independent living Dutch frail older adults. In a prospective study examining the effects of exercise and B_{12} supplementation on tHcy, MMA, and cognitive status, DeJong found no baseline or treatment effect between B_{12} therapy and neuropsychological functioning. The 17-week randomized controlled intervention study demonstrated that participants did not benefit

cognitively from B_{12} supplementation. The authors did point out, however, that the participants' lack of improvement in neuropsychological functioning may have been an indication of the B_{12} deficiency not being severe enough, the degenerating process not being developed far enough, or the supplementation trial period not being long enough. They stated that subtle neuropsychological improvements may be detected only with more sensitive tests or in more deficient patients. Overall, while longitudinal research has not been able to confirm a causal relationship between vitamin B_{12} deficiency and cognitive impairment and has found some evidence opposing a relationship between B_{12} and cognition, there remains the possibility that vitamin B_{12} deficiency does cause reversible cognitive impairment in some older adults.

Comparison of the current study with previous studies:

Despite the fact that existing research widely demonstrates that hematological abnormalities normalize within a few months of B_{12} treatment therapy, much remains unknown regarding the role of B_{12} in cognitive impairment in late life and the possibility for reversal of cognitive symptoms through B_{12} supplementation. No single study has yet prospectively examined the effects of B_{12} supplementation in a large, community population of older adults and allowed substantial time for cognitive improvements, as measured using a variety of reliable and valid neuropsychological measures, to take place.

In the current study, we document the presence and degree of vitamin B_{12} deficiency and cognitive impairment in a large sample of older adults not drawn from a special population. We focus largely on the role of MMA and associations between elevated MMA. MMA is of special interest as it has been suggested as the most specific indicator of B_{12} deficiency, particularly given recent folate fortification of food in the United States. The supplementation trial period for the study was twelve months in length, one of the longest to date. As previously presented,

researchers have theorized that deficient B_{12} levels exert their negative effects through the build up of MMA and tHcy, which in turn can lead to changes to body tissues at the cellular level, including hypomethylation and neurotoxicity (Bottiglieri, 1996). Because such cellular changes may take time, the period of twelve months was chosen to allow for potentially slow neuronal and subsequent neurological and cognitive recovery following B_{12} supplementation. We were thus able to examine potential treatment effects that may occur over an extended period of time. Indeed, as previously noted, some past researchers have theorized that their failure to find a positive treatment effect of B_{12} supplementation on cognition was due to an inadequate length of B_{12} supplementation (e.g., DeJong et al., 2001).

Furthermore, as opposed to previous studies, the population in the current study was not identified as cognitively impaired using gross screening instruments. Rather, well-validated neuropsychological measures were implemented to identify and measure the presence and progression of cognitive and psychological symptoms. Persons with a span of cognitive deficits, ranging from not-at-all impaired to seriously impaired, were included in the study, thus allowing for the analysis of possible treatment effects at all levels of symptom severity and progression.

Summary and specific aims:

Clearly a relationship exists between vitamin B_{12} and overall health. Researchers have documented both hematologic and neurologic deficits as being associated with deficient levels of vitamin B_{12}. Research shows that deficits result when vitamin B_{12} is impaired in its function as a coenzyme, in which B_{12} works to break down homocysteine, MMA, and synthesize SAM to be used in the creation and integrity of myelin. Although the exact mechanism by which B_{12} exerts its influence in maintaining cognitive and physical function is not entirely understood, it does

appear that supplementation of B_{12} in persons with elevated metabolites may bring about positive changes in cognitive and mental well-being.

The existing literature and research into the relationship between B_{12} and cognitive functioning is exciting in that it gives hope that cognitive deficits occurring in combination with deficits in vitamin B_{12} level can be reduced or even alleviated with vitamin B_{12} supplementation. Vitamin B_{12} deficiency may in fact be a reversible dementia! However, much still remains unknown regarding where cognitive deficits arise in conjunction to B_{12} deficiency, how these cognitive deficits are brought about, and whether B_{12} treatment, regardless of the mechanism of change, can create long-term cognitive and functional improvement. If such variables were better understood, it may become possible to develop more finely tuned assessment measures for the presence of vitamin deficiency-related cognitive decline. Additionally, effective treatments for people struggling with cognitive losses related to vitamin deficiency could be established. Finally, through better understanding of B_{12} and cognition, parameters for vitamin supplementation could be created, in turn preventing nutrient-deficiency related dementias and functional impairment among older adults from ever developing.

To aid in the accomplishment of such goals, the current project was designed to assess the relationship between vitamin B_{12} deficiency, MMA and tHcy elevations, cognitive losses, and recovery from cognitive deficits through B_{12} supplementation. Specifically, chapter three addresses the following aims:

1. Documentation of cross-sectional differences among a community-dwelling group of older adults in vitamin B_{12} status and cognition.

2. Investigation of relationships between serum vitamin B_{12} deficiency, elevated MMA, tHcy, and cognition in order to further clarify the mechanism in which B_{12} deficiency exerts influence on neurological function.
3. Investigation of relationships between indicators of vitamin B_{12} deficiency and specific domains of cognitive processing, including memory, attention, processing speed, reaction time, verbal fluency and visual abstract reasoning, in order to identify cognitive areas most sensitive to B_{12} deficiency.

Chapter four presents the outcome of a twelve month B_{12} replacement study and the following aims are addressed:

1. Longitudinal assessment of vitamin B_{12} supplementation and its effects on cognition in B_{12}-deficient older adults.
2. Investigation of cognitive domains most responsive to B_{12} supplementation through the measurement of a variety of domains including memory, attention, processing speed, reaction time, verbal fluency and visual abstract reasoning.

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CHAPTER 3

ELEVATED METHYLMALONIC ACID IS RELATED TO COGNITIVE IMPAIRMENT IN OLDER ADULTS ENROLLED IN AN ELDERLY NUTRITION PROGRAM[1]

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ABSTRACT

Purpose: Vitamin B_{12} status has been linked to cognitive impairment among older adults. Methylmalonic acid (MMA) may be the most sensitive index of cognitive impairment because it is a sensitive marker of B_{12} status.

Design and Methods: In a cross-sectional study of cognitive ability in B_{12}-deficient versus B_{12} adequate older adults, participants were classified as B_{12}-deficient based upon both a strict definition of B_{12} deficiency and according to elevated MMA levels. The contributions of different indices of B_{12} status, including serum B_{12}, MMA and tHcy, as well as additional biochemical and demographic variables, were measured in relation to cognitive functioning on neuropsychological tasks of reaction time, memory, processing speed, attention, verbal fluency and non-verbal reasoning.

Results: Vitamin B_{12} deficiency as measured by elevated MMA levels appeared to be the most sensitive marker of cognitive impairment and appeared to contribute unique variance to cognitive measures after controlling for biochemical variables. Demographic variables, particularly education and age, were more strongly associated with cognitive measures than was MMA.

Implications: MMA is an independent predictor of cognitive impairment associated with vitamin B_{12} deficiency in community dwelling older adults. Thus, reducing plasma MMA concentrations by administering B_{12} supplements may provide protection against cognitive decline in this and other elderly populations.

Keywords: vitamin B_{12}, homocysteine, methylmalonic acid, cognitive function, aging

INTRODUCTION

Vitamin B_{12} deficiency is common in the geriatric population, with prevalence rates ranging between 3 and 40% for Americans age 65 and older (Stabler, Lindenbaum & Allen, 1997; Baik & Russell, 1999). Research is increasingly demonstrating an association between low serum B_{12} levels and neurologic dysfunction, even in the absence of megaloblastic anemia (Carmel et al., 1995; Lindenbaum et al., 1988; Saperstein et al., 2003). Organic mental change, including dementia and/or cognitive impairment, has also been linked to B_{12} deficiency in both healthy older adults (Bohnen, Jolles & Degenaar, 1992; Goodwin, Goodwin & Garry, 1983; Kristensen, Gulmann, Christensen, Ostergaard & Rasmussen, 1993; La Rue et al., 1997; Riggs, Spiro, Tucker & Rush, 1996) and impaired older adults (Martin, Francis, Protetch & Huff, 1991) but cognitive effects remain a poorly understood correlate of poor B_{12} status. Different researchers have suggested that persons with low serum vitamin levels may have difficulty when fast and accurate processing of information is required, for example on tasks of processing speed (Bohnen et al., 1992; Jelicic, Jonker & Deeg, 2001), but be efficient in utilizing preexisting knowledge structures (Wahlin, Wahlin, Winblad & Backman, 2001). Others have suggested that B_{12} deficiency is related to impairments on global measures of cognitive functioning such as the MMSE (Bernard, Nakonezny & Kashner, 1998; Miller et al., 2003) or specific domains including constructional praxis (Riggs et al., 1996; Duthie et al., 2002), attention (Bohnen et al., 1992), and verbal fluency (Wahlin et al., 2001).

Subclinical B_{12} deficiency may be related to deficits in cerebral function and/or cognition in older adults (Carmel, 1996; Lindenbaum et al., 1988; Martin et al, 1991; van Asselt et al., 2001). This condition is characterized by low to low-normal plasma vitamin B_{12} concentrations with elevated biochemical indicators of B_{12} deficiency, including serum methylmalonic acid

(MMA) and/or total homocysteine (tHcy) concentrations, in the absence of overt hematologic or neurologic abnormalities.

Elevated serum levels of tHcy and MMA are useful indicators of vitamin B_{12} deficiency (Savage, Lindenbaum, Stabler & Allen, 1994). Vitamin B_{12} is a cofactor in the enzymatic reaction converting methylmalonyl-coenzyme A (CoA) to succinyl-CoA. A deficiency in vitamin B_{12} leads to an increase in intracellular and serum methylmalonyl-CoA and its metabolic product, MMA. B_{12} is also a necessary component in the synthesis of methionine from tHcy, utilizing a methyl group from methyl folate. Therefore, either B_{12} or folate deficiency leads to the elevation of tHcy (Stabler, 1988). Serum MMA concentration is currently the preferred metabolic indicator of vitamin B_{12} status because it is more specific since folate deficiency also elevates tHcy levels (Baik & Russell, 1999; Dharmajan, 2003; Morris, Jacques, Rosenberg & Selhub, 2002; Savage et al., 1994).

The need exists to better understand the cognitive effects of B_{12} deficiency so that patients can be identified and treated. It is now known that hematologic and neurological symptoms often do not occur together in vitamin B_{12} deficiency (Baik & Russell, 1999; Lindenbaum et al., 1998). Additionally, it is clear that subtle B_{12} deficiency can exist without the presence of marked clinical symptomatology. Thus, clinicians can no longer rely on classic clinical syndromes, such as megaloblastic anemia, to indicate B_{12} deficiency and potentially related cognitive losses. The importance of understanding and detecting cognitive symptoms related to vitamin B_{12} deficiency is imperative because the cognitive symptoms may be irreversible when vitamin B_{12} deficiency goes untreated (Healton et al., 1991; Holleland et al., 1999).

We have previously shown that B_{12} deficiency documented by elevations in serum MMA was correlated with poor performance on a global cognitive task (Johnson et al., 2003). The major purpose of this study was to assess the relationship between cognitive performance and B_{12} deficiency using detailed neuropsychological testing in a large sample of community dwelling older adults. We report cross-sectional findings of an ongoing project evaluating relationships between low B_{12}, elevations in MMA and tHcy, cognition and potential treatment effects of B_{12} supplementation on cognitive performance in older adults.

DESIGN and METHODS

Subjects

All questionnaires and procedures were approved by the University of Georgia Institutional Review Board on Human Subjects, the Georgia Department of Human Resources, and the University of Colorado Multiple Institutional Review Board. One hundred fifty participants enrolled in an Older American Nutrition Program (OANP) in one of six senior centers throughout northeast Georgia were recruited for the current project. Written consent was obtained from all participants. Of the originally recruited 150 participants, eighteen were mentally or physically unable to complete neuropsychological testing and seven refused to participate in cognitive testing. One participant was unable to provide a blood sample, and thus was not included in statistical analyses. Additionally, following laboratory analysis, eight participants with serum vitamin B_{12} levels in the 90^{th} or higher percentile based upon age and race were excluded since high B_{12} levels can be markers of liver failure (Baker, Frank & DeAngelis, 1987; Ermans, Vlasveld & Lindemans, 2003), which can lead to impaired cognitive functioning (Pantiga, Rodrigo, Cuesta, Lopez & Arias, 2003; Tartar, Sandford, Hays, Carra &

Van Thiel, 1989). Thus, 116 participants were included in the study. Participants were paid $25.00 for their participation in the project and, in addition to individual incentives, all participating senior centers received a donation of $400.00 to support the internet connection needed for neuropsychological testing.

Laboratory Techniques

Participants were not asked to fast before blood collection due to their advanced age and possible frailty. Complete blood counts and serum creatinine concentrations were determined by a local clinical laboratory (SmithKline-Beecham Clinical Laboratories, Atlanta, GA). Blood samples for the serum folate and vitamin B_{12} analyses were frozen at -70^{o} C in cryogenic vials with minimal air space (Nalgene Brand Products, Rochester, NY) until they were analyzed. Analyses for serum vitamin B_{12} and serum folate were performed with a radioassay (Quantaphase II Vitamin B_{12} /Folate Radioassay; Bio-Rad, Richmond, CA) (Gunter, Lewis & Koncikowski, 1996). Serum MMA, tHcy, serum 2-methylcitric acid, and cystathionine were analyzed by capillary gas chromatography-mass spectrometry (Allen et al., 1993; Stabler, Marcell, Podell, Allen & Lindenbaum, 1986; Stabler et al., 1988). The previously determined normal ranges were 73-271 nmol/L for MMA (Allen et al., 1993), 5.4-13.9 μmol/L for tHcy (Stabler et al., 1999), and 60-228 nmol/L for 2-methylcitric acid (Stabler et al., 1999). No participants were folate deficient (serum folate < 6.8 nmol/L, Wright et al., 1998).

Definition of vitamin B_{12} deficiency

Vitamin B_{12} deficiency was operationalized in four different ways. First, vitamin B_{12} deficiency was defined according to a strict classification criteria of serum B_{12} levels <257 pmol/L, MMA >271 nmol/L and serum MMA > serum methylcitric acid (Stabler et al., 1999). Concentrations of methylcitric acid that are greater than the serum MMA level are indicative of

renal insufficiency rather than vitamin B_{12} deficiency. Secondly, vitamin B_{12} deficiency was defined by the presence of elevated serum MMA alone (high MMA levels > 271 nmol/L; normal MMA ≤ 271nmol/L). Thirdly, vitamin B_{12} deficiency was based on elevated tHcy levels alone (high tHcy levels > 13.9 umol/L; normal tHcy ≤ 13.9 umol/L), since confounding folate deficiency was not present in this population. Finally, serum B_{12} levels alone irregardless of metabolite levels was used as a marker for vitamin B_{12} deficiency (low serum B_{12} < 257 pmol/L; normal serum B_{12} ≥ 257 pmol/L).

Serum creatinine and folate were measured to examine potentially confounding variables in the relationships between MMA and tHcy with cognition. Elevated serum creatinine (>1.4 mg/dl) is indicative of renal failure and MMA and tHcy levels have been shown to be elevated in the absence of B_{12} deficiency due to chronic renal failure (Allen, 1993; Snow, 1999). Thirteen study participants (11.2%) were found to have elevated creatinine levels; thus, it was controlled for in statistical analyses.

Protocol

Questionnaires were administered by trained interviewers to collect self-reported information on diet, health, depression, cognition, and clinical signs of vitamin B_{12} deficiency. The 15-item Geriatric Depression Scale (GDS) was used to determine possible depression (Yesavage et al., 1983). This questionnaire has been validated for use in an older adult population. Scores greater than eight suggest depression.

Cognitive functioning was measured using the Cognitive Stability Index (CSI; Headminder, 2001a), an innovative, web-based series of ten neurocognitive subtests sensitive to changes in central nervous system functioning (Headminder, 2001b). Work from our laboratory has demonstrated the CSI as a sensitive marker of differences in cognitive functioning between

independent dwelling and assisted living older adults (Petrella, Cress & Miller, 2003). The CSI is a valid and reliable measure of cognitive performance with criterion-related validity and concurrent validity to traditional face-to-face tests of cognition including the Wechsler Adult Intelligence Scale-III, Stroop Test, Wechsler Memory Scale-III, Trail Making Test A and B, and the Wechsler Abbreviated Scale of Intelligence (Headminder, 2001b). The ten subtests of the CSI measured four empirically derived cognitive factors of reaction time (RT), processing speed (PS), memory (MEM), and attention (ATT). Normative data on these four factors has been compiled for adults aged 18-90 across sex, race, and educational background (Erlanger, Feldman, Theadoracopulos & Kaplan, 2000; Headminder, 2001b). The CSI was administered by trained interviewers on a web-connected laptop at each of the six senior centers in the study. Total administration time was approximately 45 minutes.

Two additional neuropsychological tests were hand administered to all participants. The Matrix Reasoning (MR) subtest of the WASI was included to assess nonverbal reasoning, and the Controlled Oral Word Association Test (COWAT; Benton & Hamsher, 1976) was administered to assess verbal fluency. Both tasks have been interpreted as reflecting prefrontal executive control functioning (Daigneault, Braun & Whitaker, 1992; Lezak, 1995). The tasks and metrics used to quantify cognitive performance are listed in Table 3.1.

Statistics

All analyses were conducted using SPSS 11.0 (Chicago, IL). Results are shown as means ± SDs. Normal distributions of data were checked by using the Kolmogorov-Smirnov-test. Data were log transformed to approximate normal distributions where necessary. Bivariate correlations were analyzed using the Pearson correlation coefficient to identify associations among normally distributed variables. Differences in demographic variables and cognitive

variables were assessed between deficient and non-deficient groups based on each of the four definitions of vitamin B_{12} deficiency: strict B_{12} deficiency, high MMA, high tHcy, and low serum B_{12} levels alone. The independent-sample *t* test was used for continuous variables and the chi-square analysis for dichotomous variables. The Mann Whitney Test was used to analyze non-parametric data. A *p* value of $\leq .05$ was considered significant. Multiple linear regression analyses were then used to build statistical models describing the relationships between elevated MMA status (independent variable) and each of the cognitive measures (dependent variables) before and after adjustment for confounding demographic and biochemical variables.

RESULTS

Subjects

One hundred sixteen participants were included in the study. See Table 3.2 for demographic information. As found in previous studies within this population, participants reported having many impairments (Johnson et al., 2003). Approximately 29% of the sample described their overall health as fair (n=34) and 7% described it as poor (n=8). Participants reported having a mean of 5.5 (±3.2) illnesses and approximately half of the subjects were taking over 4 prescription medications daily. Approximately 11% (n=14) had a meal delivered to their home. Depression was not frequent in the sample as only 3 persons (2.6%) were classified as potentially depressed according to the GDS.

Laboratory Data

The laboratory variables are shown in Table 3.3. There were 17 individuals (14.7%) who could be classified as B_{12}-deficient based on a strict criteria of serum B_{12} levels <257 pmol/L, MMA >271 nmol/L and serum MMA > serum methylcitric acid. Elevated MMA alone was

higher, as 29.3% (N=34) of the total sample had MMA levels > 271 nmol/L. Elevated tHcy levels were less frequent with only 11.2% (N=13) of the cohort with tHcy levels > 13.9 umol/L. Lower serum B_{12} levels were associated with higher levels of MMA ($r=-.462$, $p<0.001$) and tHcy ($r=-.287$, $p<0.005$).

Correlational Relationships

Biochemicals were found to be related to one another in the anticipated directions, with lower levels of serum B_{12} being associated with higher levels of MMA and tHcy. Age was significantly associated with MMA and all domains of cognitive processing with the exceptions of reaction time, as measured by the CSI, and verbal fluency, as measured by the COWAT. Serum B_{12} alone was not linearly related to any domain of cognitive processing. The metabolites MMA and tHcy appeared to be more sensitive to cognitive ability, as MMA was inversely correlated with participants' performance on the COWAT ($r = -.284$, $p<.01$) and on the Matrix Reasoning subtest ($r = -.283$, $p<.01$). Homocysteine was inversely correlated with participant performance on the COWAT ($r = -.289$, $p<.01$). All correlations between indices of vitamin B_{12}, cognition variables and age are given in Table 3.4.

Group differences

As correlational analyses indicated that MMA was the biochemical index most strongly related to cognitive functioning, with MMA being significantly inversely correlated with participants' performance on both the MR subtest and COWAT, group differences in cognition were initially examined based upon differences in MMA level. Participants with high MMA levels (MMA > 271 nmol/L) were compared to those with normal MMA levels. The mean serum B_{12} level was 279 pmol/L (range 85 to 720 pmol/L) with 50% > 257 pmol/L. Demographic differences in age and race were found between those with high and normal MMA levels (Table

3.5). Those with high MMA were older (79.3 as compared to 74.5 years, t = -3.153, p< .01) and primarily white (30 White/4 Black as compared to 58 White/24 Black; chi square = 4.02, p<.05).

Those in the high MMA group performed significantly worse on several neuropsychological tasks (Table 3.5). On the CSI, they demonstrated more impairment on the memory index (MEM; t = 2.340, p<.05) and processing speed index (SPEED; t = -2.739, p<.05). Additionally, they performed more poorly on the hand administered tests of verbal fluency (COWAT; t = 2.215, p<.05) and nonverbal reasoning (MR; t = 3.172, p<.01). The differences remained after controlling for the effects of renal function. After statistically removing variance accounted for by the demographic variables of age and ethnicity, as well as creatinine, significant differences in performance remained between groups on the CSI SPEED index (F (4,109) = 12.493, p<.05) and the MR subtest (F (4,113) = 10.408, p<.01) and trends remained between groups on the CSI MEM index (F (4,109) = 7.578, p=.058) and COWAT (F (4,114) = 2.487, p=.086).

Based upon a strict definition of vitamin B_{12} deficiency, there were no demographic differences between the strictly defined B_{12}-deficient group and those without strictly defined deficiency. On neuropsychological testing, the strictly defined deficient group performed significantly more poorly on the COWAT (t = -2.319, p<.05), than the non-deficient group. This relationship remained even after controlling for the possible effects of renal function (F (2,114) = 4.588, p<.05). No significant differences on any additional neuropsychological measure were found when differentiating groups based upon the strict definition of B_{12} deficiency.

Those with high tHcy (> 13.9 μmol/L; n = 13) performed significantly worse than those with normal tHcy levels on the COWAT (t = 2.633, p< .05) and the MR subtest (t = 2.463, p<.05). These relationships weakened, however, after controlling for poor renal function

(COWAT; F (2,114) = 3.725, p = .061; MR; F (2,113) = 3.242, p=.072). No demographic differences existed between groups based on tHcy level.

No differences in any domain of cognitive functioning were found between groups based on serum B_{12} levels alone (<257 pmol/L).

MMA and cognitive functioning

Given the finding of strong relationships between MMA level and cognition, a series of four regression models was performed (Table 3.6) to study the relation of MMA and other biochemical and demographic variables with cognition measures. Before adjustment for confounding by biochemical and demographic variables (model 1), MMA was inversely correlated with 4 measures of cognitive function, as previously noted. The R^2 values for these simple regressions indicate that MMA explains between 3.7% and 8.2% (R^2 = .037 - .082) of the variance on the CSI Memory and CSI Processing Speed indices as well as the COWAT and Matrix Reasoning tests. MMA did not account for significant variance on the CSI Reaction Time and Attention indices. MMA remained inversely correlated with the CSI MEM and SPEED indices as well as the MR measure after the addition of serum vitamin B_{12} and creatinine to the model (model 2). The inclusion of the demographic variables (ie, age, ethnicity, education and gender) in the model, however, significantly attenuated the relations between MMA and cognitive measures (models 3 and 4). Correlation coefficients for MMA were largely reduced in these models compared with model 1, whereas R^2 values were 5 fold—10 fold those in model 1. These R^2 values indicate that the demographic variables explain a much higher proportion of the variance in cognitive scores than does MMA. Of the demographic variables, education was the most strongly associated, race and age ranked next, while gender was largely insignificant. In model 4, which included all demographic and biochemical variables, MMA remained

significantly correlated with only the MR subtest. Inclusion of the biochemical measures added very little to the variance explained by the demographic variables.

DISCUSSION

We have found that high levels of MMA correlate with impaired performance on a number of detailed neuropsychological tasks. Persons with high MMA levels showed greater cognitive impairment in the domains of information processing speed, memory, verbal fluency and nonverbal reasoning. These relationships remained after correcting for renal function, as measured by creatinine concentrations. Both cognitive processing speed and nonverbal reasoning remained significantly related to MMA after variance due to group demographic differences was removed. Of note, effect sizes in the study were moderate to high (d = .31 to .69). Therefore, even with our relatively small sample size, there was adequate statistical power to differentiate group differences in cognitive function based upon MMA level.

When B_{12} deficiency was defined according to our strict definition, including both low vitamin B_{12} and high MMA, only verbal fluency was indicated as being impaired in deficient participants. There were fewer subjects in this group, however, which may have impacted the results. Only a small percentage of the study sample had elevated tHcy levels (N=13). Homocysteine did appear a sensitive marker of potential cognitive decrement in the small number of participants with elevated tHcy, as significant differences on the cognitive tasks of matrix reasoning and verbal fluency were observed, with large effect sizes for the indices of CSI speed (d =.76), matrix reasoning (d = .96) and verbal fluency (d = 1.05). It is possible that CSI speed would have been statistically significant with a larger sample size given its large effect size. Statistically significant cognitive deficits did not remain after inclusion of creatinine in

regression models. This is in contrast to previous work (Nilsson, Gustafson & Hultberg, 2001; Riggs et al., 1996), and could be because this population was folate repleted due to folate fortification. The low rate of tHcy elevation observed is likely also related to the finding that no study participants were deficient in folate. In populations not receiving foods fortified with folate, tHcy likely remains a prevalent and strong indicator of cellular B_{12} deficiency and related cognitive decrement. However, this study suggests that in populations tested after fortification of food with folate, MMA may be the more sensitive marker of B_{12} deficiency and related cognitive deficits than either serum B_{12} or tHcy levels.

The study also highlighted cognitive skills including information processing speed, memory, verbal fluency and nonverbal reasoning as being most sensitive to MMA elevations. These cognitive tasks reflect fluid abilities, or tasks that are unfamiliar, speeded, attention-demanding and involve complex processing of information. Past studies have demonstrated similar cognitive areas as related to vitamin B_{12} deficiency. Wahlin and colleagues (2001), for example, demonstrated that low levels of serum B_{12} were associated with deficits in letter fluency and block design. Van Asselt (2001) found relationships between mild vitamin B_{12} deficiency and performance on fluency and reasoning tasks, as well as verbal learning tests. And, Miller et al. (2003) demonstrated negative correlations between tHcy level and performance on tasks of pattern recognition, verbal attention and incidental learning.

Support is thus mounting from the current as well as past studies for the frontal and temporal areas of the brain being most sensitive to nutrient deficiency. Frontal and temporal brain regions are related to executive abilities and memory function. Deficits in these areas have been implicated as most related to functional impairment in older adults (Grigsby, Kaye, Baxter, Shetterly & Hamman, 1998) and the presence of dementia. Thus, it is imperative that the true

cause of such nutrition related frontotemporal cognitive impairments be recognized so the deficiency can be promptly treated. This may in turn prevent functional losses and the development of dementia. A case study recently implicated vitamin B_{12} deficiency as the cause of newly acquired frontotemporal dementia in a 60-year-old man (Schreinzer, Barnas & Fischer, 2003). The dementia was treated and completely reversed after B_{12} supplementation. Vitamin B_{12} levels, including MMA levels, should be investigated in every patient undergoing cognitive evaluation.

Notably, statistically significant relations between MMA and several quantifiable measures of cognitive function were apparent in regression models, independent of serum B_{12} level and kidney function. The associations are modest, however, MMA predicted $\leq 8\%$ of the variance in cognitive measure scores. In contrast, demographic variables were found to be much stronger determinants of cognitive function scores, particularly education, race and age, similar to previous reports (Miller et al., 2003).

The practice of statistically removing meaningful covariance has been discouraged by authors who argue that by removing the variance due to substantively important variables, such as age, education and ethnicity, we are left with an uncharacterized, vestigial group with an uncertain relationship to the originally represented group (Miller & Chapman, 2001). Education, age and ethnicity, in our opinions, are not necessarily variables to be "controlled for" or eliminated from analyses, but rather features of the syndrome of vitamin B_{12} deficiency. Past research has indeed found similar demographic characteristics in persons with B_{12} deficiency (Miller et al., 2003). Findings suggest that more research into the associations between B_{12} level and demographic variables may promote greater identification of at-risk populations and more

thorough understanding of sensitive interventions to prevent and/or reduce cognitive deficits in such populations.

Nonetheless, current data suggesting MMA as a sensitive marker of B_{12} deficiency and related cognitive decrement, above and beyond that of serum B_{12} and tHcy, is supported by some research in the literature. It is well known that clinical B_{12} deficiency occurs with serum B_{12} levels in the conventionally defined normal ranges (Carmel, 1996; Lindenbaum et al., 1988; Lindenbaum, Savage, Stabler & Allen, 1990; Lindenbaum, Rosenberg, Wilson, Stabler & Allen, 1994). In a multicenter, double-blind, placebo controlled study, Naurath et al. (1995) demonstrated that vitamin supplementation, but not placebo, reduced MMA and tHcy levels in all study participants. The authors concluded that tissue deficiency of B_{12} is more common than serum B_{12} levels suggest, and is often present at normal serum levels. Other researchers have suggested, similar to the present study, that elevated MMA and tHcy, as opposed to serum B_{12} levels, are important in identifying neurological impairments related to B_{12} deficiency. For example, Lindenbaum et al. (1988) found that elevations in MMA and tHcy were elevated in patients with B_{12} deficiency and neurologic disease, even in the absence of anemia and low B_{12} levels. Again in 1990, Lindenbaum et al. documented that greater than five percent of medical patients with clear-cut hematologic or neurologic disorders caused by lack of the vitamin B_{12} had serum B_{12} values in the normal range, but unequivocally elevated concentrations of circulating metabolites. Such studies demonstrate that serum B_{12} levels are not sensitive and specific enough for reliable assignment of B_{12} status alone, and suggest that elevated metabolites, as found in the current study, may be more sensitive markers. Methylmalonic acid is extremely useful in diagnosis because elevations of MMA are highly specific to vitamin B12 metabolism and, as demonstrated in the current study, elevated MMA levels are sensitive to cognitive decrements.

Research has documented that virtually every patient who has clinical abnormalities that respond to vitamin B12 replacement has elevated MMA (Moelby, Rasmussen, Jensen & Pedersen, 1990; Savage et al., 1994; Stabler, 2001).

Findings also highlight the relatively high rate of B_{12} deficiency present among older adults living independently in rural NE Georgia. MMA was elevated in 29% of our sample, consistent with findings found in previous studies done in NE Georgia (Johnson et al., 2003), but substantially higher than more geographically generalized approximations in the literature (Morris et al., 2002; Stabler, Lindenbaum & Allen, 1997). The consistently high rate of B_{12} deficiency among independently dwelling older adults points out the need for more sensitive and active testing for B_{12} deficiency, as well as B_{12} supplementation, among elderly as it appears that this segment of the American older adult population is not receiving enough B_{12} rich foods nor benefiting from currently available foods fortified with B_{12}. It is interesting to note that no participants in the current study were found to be deficient in folate. In 1998 the FDA mandated that folic acid be added to a variety of grain products, such as flour and bread, which are available to older adults and typically incorporated into menus at places such as senior centers and Meals on Wheels programs.

Results of this study combined with past research in the literature demonstrate that high MMA is related to greater cognitive impairment in older adults. Additional research is needed to identify the mechanism or metabolic defect that may be responsible for the cognitive and neurological disorders associated with elevations in MMA. Additional research is also needed to examine possible treatment effects including gains in cognitive functioning that may be associated with B_{12} supplementation and the subsequent decline in MMA and tHcy. Cognitive performance has been shown to improve with B_{12} treatment in a study reported recently (Van

Asselt et al., 2001). The modest association between MMA concentration and cognitive function observed in the present study does suggest, however, that the effect of B_{12} vitamin supplementation may be limited. It is also possible that irreversible damage may occur if B_{12} deficiency persists for long intervals (Healton et al., 1991; Martin et al., 1991). However, high dose oral vitamin B_{12} replacement is safe and inexpensive and the decrease in tHcy seen with such treatment may have additional benefits (Johnson et al., 2003).

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TABLE 3.1
Cognitive Measures: List of Performance tasks and Metrics

Task	Measurement
Attention/Working Memory on CSI	
Number Recall	# Correct, # Errors
Number Sequencing	# Correct, # Errors
Spatial Memory/Learning on CSI	
Object Memory Recall	# Correct over four trials
Delayed Object Memory Recall	% Retained
Pictorial Incidental Learning # 2	Time, # Errors, # Omissions
Reaction Time on CSI	
Number Recognition Response	Time, # Correct, # Omissions
Opposite Number Recognition Response	Time, # Correct, # Omissions
Pictorial Incidental Learning # 1	Time, # Correct, # Omissions
Pictorial Incidental Learning # 2	Time, # Correct, # Omissions
Processing Speed on CSI	
Symbol Scanning	Time, # Correct
Pictorial Animal Decoding	# Correct, # Errors
Nonverbal Reasoning	
Matrix Reasoning Subtest	# Correct
Verbal Fluency	
Controlled Oral Word Association Test (COWAT)	# Correct

TABLE 3.2
Description of Study Population[a]

	Value
Age (y)	75.9 ± 7.7[b] (58-97)[c]
Gender	
Male	21[d] [18][e]
Female	95 [82]
Race	
White	88 [76]
Black	28 [24]
Education (y)	9.5 ± 3.4 (0-18)

[a]*n* = 116
[b]mean ± sd
[c]range in parentheses
[d]N
[e]percentage in brackets

TABLE 3.3
Biochemical Variables[a]

Variable	
Serum B_{12}	451 ± 169[b] (101-979)[c]
% < 258 pmol/L	28.4 [33][d]
% < 258 pmol/L & MMA > 271 nmol/L	14.7 [17]
Serum Folate	20 ± 11 (6-72)
% < 6.8 nmol/L	0 [0]
Serum MMA	273 ± 200 (104-1972)
% > 271 nmol/L	29.3 [34]
Serum tHcy	10.4 ± 3.2 (5.4-23.5)
% > 13.9 µmol/L	11.2 [13]
Creatinine	1.1 ± 0.4 (0.6-4.6)
% >1.4 mg/dl	11.2 [13]

[a]N = 116
[b]mean ± sd
[c]range in parentheses
[d]N in brackets

TABLE 3.4
Intercorrelations between vitamin B_{12} indices, cognition and demographic variables in the older adult population

Variable	1	2	3	4	5	6	7	8	9	10
1. Serum MMA	--	-.462**	.577**	.119	-.148	-.119	-.030	-.284**	-.283**	.396**
2. Serum Cbl		--	-.287**	.069	.032	.061	.036	.109	-.048	.001
3. Serum tHcy			--	.138	-.108	-.080	-.052	-.289**	-.165	.175
4. CSI SPEED				--	-.575**	-.566**	-.486**	-.373**	-.497**	.389**
5. CSI MEM					--	.516**	.276**	.372**	.420**	-.413**
6. CSI ATTN						--	.425**	.312**	.570**	-.366**
7. CSI Reaction Time							--	.301**	.323**	-.144
8. COWAT								--	.480**	-.139
9. Matrix Reasoning									--	-.385**
10. Age										--

*p<.05, **p<.01

TABLE 3.5
Differences between groups as differentiated by high vs. normal MMA level[a]

Variable	High MMA (N=34)	Normal MMA (N=82)	p	p*	p**	*d*
Demographics						
Age (y)	79.3 ± 6.5[b]	74.5 ± 7.8	<0 .01			
Race (% white)	88	71	< 0.05			
Gender (% male)	21	17	NS			
Education (y)	9.9 ± 3.5	8.7 ± 2.8	NS			
Cognitive Scores						
CSI ATTN[c]	4.7± 3.1	5.7 ± 3.4	NS	NS	NS	.48
CSI MEM[d]	5.0 ± 1.6	5.8 ± 1.7	<0.05	<0.05	.057	.47
CSI SPEED[e]	-14.0 ± 9.8	-10.3 ± 6.0	<0.05	<0.05	<0.05	.38
CSI Reaction Time[f]	-1.0 ± 0.20	-.93 ± 0.17	NS	NS	NS	.31
COWAT	17.8 ± 7.9	22.1 ± 10.2	<0.05	<0.05	NS	.48
Matrix Reasoning	5.7 ± 5.1	9.9 ± 7.0	<0.01	<0.01	<0.01	.69

[a]N = 116
[b]Mean ± SD
[c]N=102
[d]N=112
[e]N=111
[f]N=105
*corrected for creatinine
**corrected for age, race, creatinine

TABLE 3.6
Multiple linear regression models for MMA (independent variable) versus cognitive measure score (dependent variable)[a]

	Model 1			Model 2			Model 3			Model 4		
	R^2	Coefficient	P	R^2	Coefficient	P	R^2	Coefficient	P	R^2	Coefficient	P
CSI Reaction Time	.005	-.003 ± .038[b]	NS	.013	-.004 ± .043	NS	.147	-.008 ± .041	NS	.159	-.004 ± .046	NS
CSI MEM	.037	-.797 ± .341	<.05	.060	-1.02 ± .391	<.05	.253	-.340 ± .336	NS	.296	-.623 ± .371	NS
CSI SPEED	.049	.117 ± .049	<.05	.063	.124 ± .055	<.05	.417	.006 ± .044	NS	.420	.006 ± .049	NS
CSI ATTN	.008	-1.01 ± .726	NS	.047	-1.50 ± .834	NS	.289	-.315 ± .705	NS	.346	-.688 ± .784	NS
COWAT	.041	-4.34 ± 1.96	<.05	.085	-2.35 ± 2.16	NS	.308	-2.86 ± 1.87	NS	.333	-1.36 ± 2.04	NS
Matrix Reasoning	.082	-4.24 ± 1.36	<.01	.111	-4.18 ± 1.51	<.01	.419	-3.01 ± 1.19	<.05	.470	-.451 ± .159	0.01

[a]Multiple linear regression models are as follows: Model 1 = MMA group alone; model 2 = model 1 + ln(serum B_{12}) + ln(creatinine); model 3 = model 1 + age + gender + race + education; Model 4 = model 2 + model 3
[b]mean ± standard deviation

CHAPTER 4

THE EFFECT OF VITAMIN B_{12} SUPPLEMENTATION ON THE COGNITIVE FUNCTION OF OLDER ADULTS WITH ELEVATED METHYLMALONIC ACID LEVELS[1]

[1] Lewis, M. S., Miller, L. S., Johnson, M. A., Dolce, E. B., Allen, R. H. & Stabler, S. P. To be submitted to *Biological Psychology*.

ABSTRACT

Cognitive deficits on neuropsychological tasks have been found to be associated with methylmalonic acid (MMA) elevations due to vitamin B_{12} deficiency in older adults. Improvement of cognitive deficits through B_{12} replacement therapy may be possible in these persons. In a double blind longitudinal study, those with high MMA levels underwent oral B_{12} replacement therapy (1000 μg pills) with measurement of serum vitamin levels. Neuropsychological test performance was measured at three time points over twelve months. Participants with high baseline MMA levels showed cognitive decrement relative to those with normal MMA levels in the domains of memory, verbal fluency and abstract reasoning. Following B_{12} supplementation, those responding metabolically to treatment demonstrated marked improvements in memory and abstract reasoning relative to the placebo group. Reducing serum MMA concentrations through B-vitamin supplementation may provide protection against cognitive decline as well as reverse potential cognitive deficits related to vitamin B_{12} deficiency in this and other elderly populations.

Keywords: vitamin B_{12}, methylmalonic acid, homocysteine, cognitive function, aging

INTRODUCTION

Vitamin B_{12} deficiency commonly occurs in older adults, with prevalence rates estimated at approximately 15% among geriatric populations (Stabler, Lindenbaum & Allen, 1997; Rajan et al., 2002). B12 deficiency has been linked to both hematologic and neurologic deficits (Naurath et al., 1995; Lindenbaum et al., 1988; Healton, Savage, Brust, Garrett & Lindenbaum., 1991). Researchers have now added impaired cognition to the list of ills associated with vitamin B_{12} deficiency (van Asselt, Pasman, van Lier & Vingerhoets, 2001; Wahlin, Wahlin, Winblad & Backman, 2001). More specifically, elevations in the vitamin B_{12} metabolites MMA and tHcy, as opposed to depleted serum B_{12} levels alone, are now being suggested as most strongly related to impaired cognitive processing in multiple cognitive domains in older adults. This may be due to metabolites being sensitive to neuronal changes at the cellular level associated with decrements in vitamin B_{12} (Baik & Russell, 1999; Dharmarajan, 2003; Morris, Jacques, Rosenberg & Selhub, 2002).

Previous research has suggested that elevations in MMA account for unique variance in the relationship between impaired cognition and B_{12} deficiency (Lewis, Miller, Johnson, Stabler & Giumelli, 2002). Persons with elevated MMA levels were found to perform more poorly than those with normal MMA levels on tasks of memory, processing speed, verbal fluency and nonverbal reasoning. Miller et al. (2003) found comparable relationships between elevations in the metabolite tHcy and cognitive impairment in similar cognitive domains, including the global modified Mini Mental State Examination, picture-association, verbal attention and pattern recognition tasks. MMA levels have been suggested as being the preferred indicator of vitamin B_{12} status, however, as they have been found to represent a metabolic change that is highly specific to deficiency of vitamin B_{12} (Baik & Russell, 1999).

Vitamin B_{12} deficiency, and more specifically elevations in the metabolites MMA and tHcy, is tied to cognitive decrements in older adults. Thus, research into the benefits of supplementation, or B_{12} replacement therapy, appears justified. It has previously been found that B_{12} supplementation corrects the metabolic abnormalities associated with B_{12} deficiency (i.e., elevated MMA, tHcy concentrations and megaloblastic anemia; Bjorkegren & Svaarsudd, 1999; Naurath et al., 1995). Vitamin B_{12} replacement has also been shown to improve (Lindenbaum et al., 1988) or halt (Saperstein et al., 2003) some neurologic and cognitive deficits associated with B_{12} deficiency, such as parasthesia, ataxia, memory loss and limb weakness.

Findings are mixed regarding the efficacy of B_{12} supplementation on cognitive processing, however. An intervention trial recently showed that vitamin B_{12} replacement improved cognitive performance and abnormalities on electroencephalogram in vitamin B_{12}-deficient seniors (van Asselt et al., 2001). This finding adds to previous research which has suggested improvements in some mental status scores can occur in B_{12}-deficient seniors with a short duration of pre-treatment mental symptoms (Martin, 1992), or in older adults with predominantly pre-treatment memory problems as opposed to more severe forms of cognitive impairment or dementia (Eastley, Wilcock & Bucks, 2000). Vitamin B_{12} replacement therapy has not been proven effective in improving mental symptoms in persons with long standing dementia (Carmel et al., 1995). Additional research has demonstrated B_{12} supplementation therapy as also being ineffective in bringing about cognitive enhancement among community dwelling adults with varying levels of B_{12} deficiency (DeJong et al., 2001; Kwok et al., 1998). Thus, the effects of B_{12} replacement therapy on cognitive function remain unclear.

To further investigate the effects of B_{12} supplementation on cognition in B_{12}-deficient community dwelling older adults, we performed a double blind treatment outcome study and

determined the effects of B_{12} supplementation on neuropsychological test performance in older adults with MMA elevation. We hypothesized that persons with elevated MMA levels, indicative of B_{12} deficiency, would show significant cognitive decrements and that these cognitive decrements would moderately respond to B_{12} supplementation. Previous research has indicated additional factors may contribute to the effectiveness of B_{12} replacement therapy, such as length and severity of both cognitive and B_{12} decrements (Martin et al., 1992) as well as various demographic variables (Lewis et al., 2002; Miller et al., 2003). Thus, we further hypothesized that, even in the absence of large group treatment effects, individual improvements in cognition associated with B_{12} supplementation would be evident.

DESIGN and METHODS

Subjects

The University of Georgia Institutional Review Board on Human Subjects, the Georgia Department of Human Resources and the University of Colorado Multiple Institutional Review Board approved all questionnaires and procedures used in this study. Participants were recruited from the Older Americans Act Nutrition Program at six senior centers in Northeast Georgia. Written consent was obtained from all participants.

For the purposes of this study, vitamin B_{12} deficiency was defined as serum MMA > 271 nmol/L at baseline prior to assignment to supplements. After baseline testing, participants were placed in either the B_{12}-adequate group (MMA < 271 nmol/L, n = 103) or the B_{12}-deficient group (MMA $\geq$ 271 nmol/L, n = 45). To ensure treatment compliance, deficient participants were made aware of their deficiency state by the phlebotomist. In addition, the physicians of the deficient participants were notified. None of the deficient participants elected to receive medical

treatment in place of study participation. Psychology researchers and staff remained blind to this information. Those in the deficient group received tablets containing 1,000 mcg vitamin B_{12} and were instructed to take one tablet daily.

A randomized, double blind, placebo-controlled design was used for the vitamin B_{12} adequate group. Participants were assigned to receive either 0 mcg (placebo, n=37), 25 mcg, or 100 mcg tablets. All participants were instructed to take one tablet daily. Only those participants in the treatment and placebo groups were included in the current study. Of the original 82 participants included in both the treatment and placebo study groups, thirty persons attrited for various reasons during the twelve month treatment phase. Nine quit the study following baseline assessment, three died, five were excluded for medical reasons (hospitalized, nursing home), twelve were unable to be reached due to extended vacation or change of address, and four were mentally unable to complete neuropsychological testing. Additionally, one person was found to be a statistical outlier and excluded from statistical analyses (Cooks D = .270 at Time 1 testing, .268 at Time 3 testing). Handwritten notes by the study examiner confirmed that this person was distracted and demonstrated marked difficulty using the computerized testing procedure during data collection. Nine persons in the treatment group did not respond metabolically to the B_{12} supplement and thus were excluded from statistical analyses. Possible reasons for non-responders included poor compliance, poor gastric function and poor renal clearance of MMA. These factors may have contributed to the poor metabolic response to B_{12} supplementation in these participants. Thus, in total, 21 participants were included in the final placebo group and 18 in the treatment group. Participants were paid $25.00 following each wave of study completion and, in addition to individual incentives, all participating senior centers received a donation of $400.00 for their involvement. Participant characteristics are given in Table 4.1.

Design

Time 1 assessments were performed in the six senior centers January through April of 2001. After approximately 3 months of supplementation, time 2 testing was completed between May and August of 2001. Time 2 testing involved the same methods for blood draw, questionnaires, and cognitive screening as time 1. Percent compliance was calculated using pill counts. As research suggests cognitive improvements with vitamin B_{12} treatment likely take place at a cellular level (Bottiglieri, 1996) and may take several months to become apparent, placebo and treatment groups continued supplementation after time 2 testing with time 3 testing occurring from December 2001 through March 2002, approximately twelve months after supplementation was initiated at baseline. Time 3 testing again involved the same methods for blood draw, questionnaires, and cognitive screening. Again, percent compliance was calculated using pill counts.

Laboratory Techniques

Participants were not asked to fast before blood collection due to their advanced age and possible frailty. Complete blood counts including serum creatinine concentrations were conducted by a local clinical laboratory (SmithKline-Beecham Clinical Laboratories, Atlanta, GA). Blood samples for the serum folate and vitamin B_{12} analyses were frozen at -70^{o} C in cryogenic vials with minimal air space (Nalgene Brand Products, Rochester, NY) until they were analyzed. Analyses for serum vitamin B_{12} and serum folate were performed with a radioassay (Quantaphase II Vitamin B_{12} Folate Radioassay; Bio-Rad, Richmond, CA) (Gunter et al., 1996). Serum MMA, tHcy, serum 2-methylcitric acid, and cystathionine were analyzed by capillary gas chromatography-mass spectrometry (Stabler 1986; Stabler 1988; Allen 1993; Stabler 1993). The previously determined normal ranges were 73-271 nmol/L for MMA (Allen, 1993), 5.4-13.9

μmol/L for tHcy (Stabler, 1999), and 60-228 nmol/L for 2-methylcitric acid (Stabler, 1999). No participants were folate deficient (serum folate < 6.8 nmol/L, Wright et al., 1998).

Protocol

Questionnaires were administered by trained interviewers to collect self-report information on diet, health, depression, cognition, and clinical signs of vitamin B_{12} deficiency. The 15-item Geriatric Depression Scale (GDS) was used to determine possible depression (Yesavage et al., 1983). This questionnaire has been validated for use in an older adult population. Scores greater than eight suggest depression.

Cognitive functioning was measured using the Cognitive Stability Index (CSI; Headminder, 2001a), an innovative, web-based series of ten neurocognitive subtests sensitive to changes in central nervous system functioning (Headminder, 2001b). The CSI is a valid and reliable measure of cognitive performance with criterion-related validity and concurrent validity to traditional face-to-face tests of cognition including the Wechsler Adult Intelligence Scale-III, Stroop Test, Wechsler Memory Scale-III, Trail Making Test A and B, and the Wechsler Abbreviated Scale of Intelligence (Headminder 2001b). The ten sub-tests of the CSI have been shown to measure four empirically derived cognitive factors of reaction time (RT), processing speed (PS), memory (MEM), and attention (ATT) in adult populations. However, as these factors were not found in statistical analyses to hold together in the current study's population, with the exception of the RT factor, individual subtests were utilized in data analyses. The CSI subtests and metrics used to quantify cognitive performance are listed in Table 4.2. Normative data on the ten subtests has been compiled for adults aged 18-90 across sex, race, and educational background (Headminder, 2001b). The CSI was administered by trained interviewers on a web-

connected laptop at each of the six senior centers in the study. Total administration time was approximately 45 minutes.

Two additional neuropsychological tests were hand administered to all participants. The Matrix Reasoning (MR) subtest of the Wechsler Abbreviated Scale of Intelligence was included to assess nonverbal fluid reasoning, and the Controlled Oral Word Association Test (COWAT; Benton & Hamsher, 1976) was administered to assess verbal fluency. Both tasks have been interpreted as reflecting prefrontal executive control functioning (Lezak, 1995; Daigneault, Braun & Whitaker, 1992). Please see Table 4.2 for tasks and metrics used to quantify cognitive performance.

Statistics

Statistical analyses were conducted using SPSS 11.0 (Chicago, IL). A p-value of < 0.05 was considered significant. Cross-sectional analyses including *t* tests for continuous variables and chi-square analyses for dichotomous variables were used to determine differences at baseline between the MMA elevated, normal and attrition groups. To avoid potential Type 1 error resulting from multiple comparisons we apriori decided to examine only Baseline and Time 3 cognitive data. Paired *t* tests and chi-square analyses were used to compare time 1 and time 3 data within treatment groups. The Wilcoxon two-sample test was used to analyze non-parametric data. Difference scores were calculated for selected variables to determine changes in these variables from baseline to time 3. Difference scores were compared between groups using independent *t* tests. Data was log transformed to approximate normal distributions where necessary. The treatment intervention was analyzed using repeated measures ANOVA to examine the group differences in how various cognitive measures, as listed in Table 4.2, changed from baseline to time 3 testing.

RESULTS

Initially the attrition group was examined for meaningful differences from those participants who completed all three waves of data collection. Of the 30 persons who did not provide data post baseline collection, 15 had been originally assigned to the placebo group and 15 had been assigned to the treatment group. Independent t-tests demonstrated that no significant differences existed between the attrition group and participant group in age, educational level, self-reported health, depression level, reported illnesses, prescriptions or other reported health factors. Metabolically, the attrition group had significantly higher baseline folate levels (24.6 ± 14.1 vs. 18.3 ±12.5, $p<.05$) and B_{12} levels (536.4 ± 173.8 vs. 430.5 ± 224.9, $p<.05$) than the included participant group. This may be due in part to four attrition participants having serum B_{12} levels in the 90th or higher percentile based upon age and race at baseline. Such high levels of B_{12} have been suggested as being associated with hepatitis (Ermens, Vlasvel & Lindemans, 2003; Baker, Frank & DeAngelis, 1987). Generally, attrition appeared to be randomly distributed throughout the original sample.

The normal MMA placebo and high MMA treatment groups differed significantly at baseline in age, with the placebo group being younger (71.3 ± 5.8 vs. 77.9± 6.6; $t = -3.3$, $p<.05$) than the treatment group. No additional differences in demographic variables were observed. No participants in either study group were depressed according to testing on the GDS.

Neuropsychological scores at baseline and at follow-up Time 3 testing are shown in Table 4.3. The high MMA treatment group had lower baseline cognitive scores on most cognitive measures at Baseline testing, with significantly poorer performance on the CSI incidental learning subtest ($t = 2.10$, $p<.05$), and marginally significantly lower scores on the COWAT ($t = 1.81$, $p=.077$) and Matrix Reasoning subtest ($t = 1.95$, $p=.059$). Such findings are

consistent with results from previous studies in our laboratory (Lewis et al., 2002). Of note, when controlling for age in statistical analyses, significant and marginally significant relationships were attenuated.

The normal MMA placebo group generally remained the same in their neuropsychological test performance across time points. They did show significant improvement in their scores on the Memory Cabinet CSI subtest. Other subtest scores deviated nonsignificantly around the mean. The high MMA treatment group demonstrated significant improvement from baseline on the CSI Incidental Learning subtest (Wilcoxon $Z = -2.59$, $p < .05$) and on the Matrix Reasoning subtest ($t = -3.81$, $p<.01$). When the changes in scores were compared with those of the placebo group, the treatment participants showed significantly better rates of change on both the CSI Incidental Learning subtest [$F(1,36) = 5.07$, $p<.05$] and the Matrix Reasoning subtest [$F(1,36) = 5.78$, $p<.05$]. Those nine participants who failed to respond metabolically to vitamin B_{12} supplementation did not show significant changes in cognitive ability over time. See Figures 4.1 and 4.2 for participant scores on these measures across all three time waves. No additional between group differences were evident.

When cognitive functioning was compared between groups selected according to serum tHcy level (serum tHcy > 13.9 umol/L) and subsequently serum B_{12} level (serum $B_{12} < 350$ pmol/L), no significant group differences in cognition were evident and no significant changes across time were present.

DISCUSSION

Results of the current study support existing research demonstrating that persons with vitamin B_{12} deficiency, and more specifically MMA elevations, demonstrate greater cognitive deficits in various cognitive domains than those with normal MMA levels. Furthermore, findings

suggest that benefit to cognitive function may be obtained through B_{12} supplementation in older people with MMA elevations.

Although statistical findings in the current study are modest, older adults with high MMA demonstrated varying degrees of cognitive impairment in several domains of cognitive functioning in the current study. Although when controlling for age in statistical analyses, significant and marginally significant relationships were attenuated, the practice of removing meaningful covariance from demographic variables in statistical analyses has been discouraged by authors (Miller & Chapman, 2001). The observed advanced age of the treatment group is, in our opinions, a feature of the syndrome of vitamin B_{12} deficiency, rather than something to be controlled for in data analyses.

MMA levels, as opposed to serum B_{12} or tHcy levels, appeared to be the most sensitive indicator of cognitive decrements in the older adult population. This is consistent with previous research in our laboratory using a larger sample size (Lewis et al., 2002) in which persons with high MMA levels showed greater cognitive decrements than those with normal MMA levels on tasks of memory, processing speed, verbal fluency and abstract reasoning. Indeed, even among the small sample size of the current study, persons with high MMA levels performed significantly worse than persons with normal MMA levels on the CSI Incidental Learning subtest, as well as moderately worse on the COWAT and Matrix Reasoning subtests. When using serum B_{12} or tHcy to differentiate groups, few cognitive differences, if any, were apparent in both the current and previous research studies. While serum B_{12} did not appear to be a sensitive measure of cognitive functioning, tHcy elevations simply were not prevalent in the study population. This is likely due to recent folate fortification by the USDA of grain based food products. Homocysteine did appear a sensitive marker of potential cognitive decrement in

the small number of participants with elevated tHcy. Thus, in populations not receiving foods with folate fortification, tHcy levels likely remains a prevalent and sensitive marker of cellular B_{12} deficiency and potentially related cognitive deficit. Such findings lend support to the role of elevated metabolites as the most sensitive markers for not only tissue deficiencies of B_{12} and neurological deficits, as demonstrated in other studies (Savage et al., 1994; Moelby et al., 1990; Lindenbaum et al., 1988; Healton et al., 1991), but furthermore, as this study demonstrates, cognitive decrements. MMA may be a more prevalent marker in older adult populations, given the current folate fortification of foods in the United States.

Such findings evoke the possibility that impaired function of the enzyme methylmalonyl-CoA mutase, which is responsible for catalyzing the conversion of methylmalonyl-CoA to succinyl-CoA, does in fact contribute to cognitive dysfunction in the elderly. This finding is in contrast to some previous research suggesting no relation between concentration of serum MMA and cognitive performance (Nilsson et al, 2001; Lehmann, Gottfries & Regland, 1999). Additional research regarding the mechanism through which vitamin B_{12} deficiency exerts its negative effects on neurologic function, with an emphasis on the role of methylmalonyl-CoA mutase, appears warranted. MMA elevations alternatively, may be an indicator of impairments in transcobalamin II (TCII), the primary serum transport protein for vitamin B_{12}. (Miller et al., 2002).

Defects of TCII cause severe megaloblastic anemia, elevations in MMA and tHcy, neurologic disease, and immune deficiency in the first few months of life. However, because most of the vitamin B-12 in serum is bound to other proteins (TCI and TCIII), these patients do not manifest a low serum vitamin B_{12} concentration (Stabler, 2001). As megaloblastic anemia can be masked through adequate intakes of folic acid, a high MMA value may be the only visible

indicator in folate adequate older adults of a breakdown in the transport of serum B_{12} to the cells where it is most needed. Highly significant correlations between total plasma transcobalamin levels and long-term memory have been documented previously in the literature (Bjorksten, Dige & Nexo, 2001).

This notion of MMA as an important indicator of cognitive deficit in older adults is further supported in the current study by the finding of cognitive improvement in the high MMA treatment group, who experienced normalizing MMA levels through vitamin B_{12} replacement therapy. Indeed, it is notable that in our very small sample of older adults, some cognitive progress was made. Cognitive improvement was demonstrated on select neuropsychological tasks following a twelve month regimen of oral B_{12} supplementation in those persons with elevated baseline MMA levels who responded metabolically to B_{12} supplementation. Significant cognitive improvement in the high MMA treatment group was demonstrated on the CSI Incidental Learning subtest and the Matrix Reasoning subtest. Of note, in participants with high MMA at baseline who failed to respond metabolically to B_{12} supplementation, there was no marked improvement after twelve months on either of these subtests. Similarly, participants in the normal MMA placebo group did not demonstrate improvements across time on either of these subtests.

The normal MMA placebo group did, however, show some significant cognitive improvement over time in the current study, namely on the CSI Memory Cabinet subtest. Unlike the Incidental Learning and Matrix Reasoning tasks, however, the Memory Cabinet is a task including multiple repeated trials of similar information, and observed improvements in the placebo group may have been due to a practice effect. This explanation is a less likely rational for explaining the treatment group's improvements over time on the single trial Incidental

Learning and Matrix Reasoning subtests. Furthermore, as noted, participants who failed to respond metabolically to vitamin B_{12} supplementation did not demonstrate significant improvements on these subtests. Such a finding mitigates the likelihood that practice effects accounted for the positive changes in learning and reasoning demonstrated by the high MMA treatment group who did respond metabolically to B_{12} supplementation .

The findings highlight the cognitive domains of learning and abstract reasoning as significantly sensitive to B_{12} supplementation. Support in cross-sectional studies exists for similar domains being sensitive to vitamin B_{12} deficiency among older adults, namely those domains that require fast and accurate processing of novel information (Wahlin et al., 1991). Our research expands past findings through the use of a longitudinal design and gives hope that the very cognitive abilities showing decrement in persons with B_{12} deficiency may be enhanced through B_{12} replacement therapy.

A number of limitations were present in this study that should be noted. First, due to a high rate of study attrition as well as a number of participants who failed to respond metabolically to B_{12} supplementation, the overall sample size of the current study was considerably smaller than the number of participants included at baseline testing. The small sample size may have contributed to a number of statistical problems. These include low power and restricted ranges of the dependent variables used in the current study. Such problems potentially reduced the strength of study findings.

Additionally, given the small study population, findings may have limited generalizability to other populations. However, the sample size of 18 treatment group participants, as used in the present study, is comparable to similar prospective studies in the literature addressing the influence of B_{12} supplementation on cognition among older adults

(Kwok et al., 1998; van Asselt et al., 2000; Martin et al., 1992). Although it is a difficult process to attract and retain participants with B_{12} deficiency for studies of this sort, the fact that statistical findings have been documented in this and similar studies, not withstanding the low sample sizes and limited statistical power, underscores the importance of recognizing and treating the presence of B_{12} deficiency in older adults. By doing so, cognitive decrements in older adults may be reversed or prevented. In addition to utilizing a larger sample size, results may have increased in the current study by implementing a trial period of even longer than twelve months, so as to allow for potentially greater neuronal regeneration following B_{12} supplementation.

The current study would have greatly benefited from the inclusion of information regarding duration of participant B_{12} deficiency status and duration of cognitive symptoms. Because our study population was comprised of independently dwelling older adults recruited though community senior centers as opposed to hospitals or medical clinics, we had no independent measure of length of metabolic or cognitive deviation. Researchers have suggested that there may be a time-limited window of opportunity for effective intervention in patients with reversible dementias (Freemon & Rudd, 1982), including cognitive dysfunction related to B_{12} deficiency (Martin et al, 1992). It has been hypothesized that with longer duration of illness related to B_{12} deficiency comes a loss of ability for neuronal repair in the body (Martin et al., 1992). Thus, in future studies of this sort, information regarding length of metabolic abnormality and cognitive symptomatology, while difficult to obtain, would be beneficial and possibly clarify those participants in which a treatment effect was most probable.

Taken together, findings from this exploratory study suggest those with elevated MMA levels, as brought about by B_{12} deficiency, experience mild decrements in select domains of cognitive processing. Furthermore, these decrements may be amenable to improvement through

B_{12} replacement therapy. Existing research suggests that elevated metabolite levels, namely MMA and tHcy, can be indicative of neurological abnormalities, even in the absence of serum B_{12} deficiencies (Healton et al., 1991; Lindenbaum, 1988). We build upon this research through the demonstration that MMA elevations may also be indicative of cognitive decrements and that these decrements may respond to B_{12} supplementation. Additional research is warranted to further understand the interplay of metabolic influences on cognition and potential reversal of cognitive decrements through vitamin replacement therapy.

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TABLE 4.1
Description of Study Population

	Normal MMA - Placebo	High MMA - Treatment
No.	21	18
Age (yr)	71.3 ± 5.8[1] (63-85)[2]	77.9 ± 6.6* (69-91)
Gender		
Male	2 [10][3]	4 [22]
Female	19 [90]	14 [78]
Race		
White	14 [66]	16 [89]
Black	7 [33]	2 [11]
Education (y)	9.0 ± 3.8 (0-16)	8.9 ± 3.1 (2-16)

*Significant difference between treatment and placebo group, $p<.05$.
[1]mean ± sd
[2]range in parentheses
[3]percentage in brackets

TABLE 4.2
Cognitive Measures: List of Performance tasks and Metrics

Task	Measurement
Attention/Working Memory on CSI	
Number Recall	# Correct
Number Sequencing	# Correct
Spatial Memory/Learning on CSI	
Object Memory Recall	# Correct over four trials
Delayed Object Memory Recall	% Retained
Pictorial Incidental Learning # 2	# Correct
Reaction Time on CSI*	
Number Recognition Response	Time in seconds
Opposite Number Recognition Response	Time in seconds
Processing Speed on CSI	
Symbol Scanning	Time in seconds
Pictorial Animal Decoding	# Correct
Nonverbal Reasoning	
Matrix Reasoning Subtest	# Correct
Verbal Fluency	
Controlled Oral Word Association Test	# Correct

*Scores combined into a Reaction Time Index Score, coefficient alpha = .712

TABLE 4.3
Biochemical and Neuropsychological scores across time[1]

	Baseline		Time 3	
	Normal MMA - Placebo	High MMA - Treatment	Normal MMA - Placebo	High MMA - Treatment
Methylmalonic Acid (nmol/L)	197 ± 56	504 ± 400**	254 ± 98	256 ± 53†‡
Homocysteine (μmol/L)	8.9 ± 1.7	13.3 ± 4.7**	9.0 ± 1.9	8.2 ± 1.6†‡
Serum B_{12} (pmol/L)	448 ± 165	327 ± 165*	439 ± 185	1260 ± 572**†‡
Number Recall raw score	3.0 ± 2.2	3.1 ± 2.1	3.7 ± 2.6[2]	3.0 ± 2.2
Number Sequencing raw score	1.8 ± 1.9[2]	1.9 ± 1.5[3]	1.8 ± 1.8[2]	1.7 ± 1.8
Memory Cabinet Total raw score	20.5 ± 9.1[2]	15.3 ± 8.8	22.3 ± 8.7†	18.6 ± 9.5[3]
Memory Cabinet Delay raw score	5.0 ± 2.9[2]	3.6 ± 2.4	4.6 ± 3.4	3.6 ± 2.6[3]
Incidental Learning raw score correct	32.0 ± 4.7[2]	28.3 ± 6.0*	31.7 ± 5.3	31.7 ± 3.8†‡
Reaction Time in seconds	-1.6 ± .45	-1.7 ± .67	-1.8 ± .57	-2.0 ± .88
Symbol Scanning raw score	16.6 ± 3.7[2]	15.4 ± 3.8[3]	15.3 ± 3.1	14.1 ± 3.8
Animal Decoding raw score	13.7 ± 7.6	11.2 ± 6.7	15.5 ± 7.0	12.3 ± 6.7
COWAT raw score	22.4 ± 10.2	16.7 ± 9.3[#]	21.1 ± 9.9	16.5 ± 8.2
Matrix Reasoning raw score	10.6 ± 7.2[2]	6.4 ± 5.8[#]	9.1 ± 5.0	8.5 ± 6.0†‡

[1]n = 21 placebo, 18 treatment. [2]n=20. [3]n=17.
*Significant difference between placebo and treatment group, p<.05. **p<.01.
[#]Trend towards significant difference between placebo and treatment group
†Significant difference from baseline, p<.05.
‡Significant group difference in change of scores, p<.05.

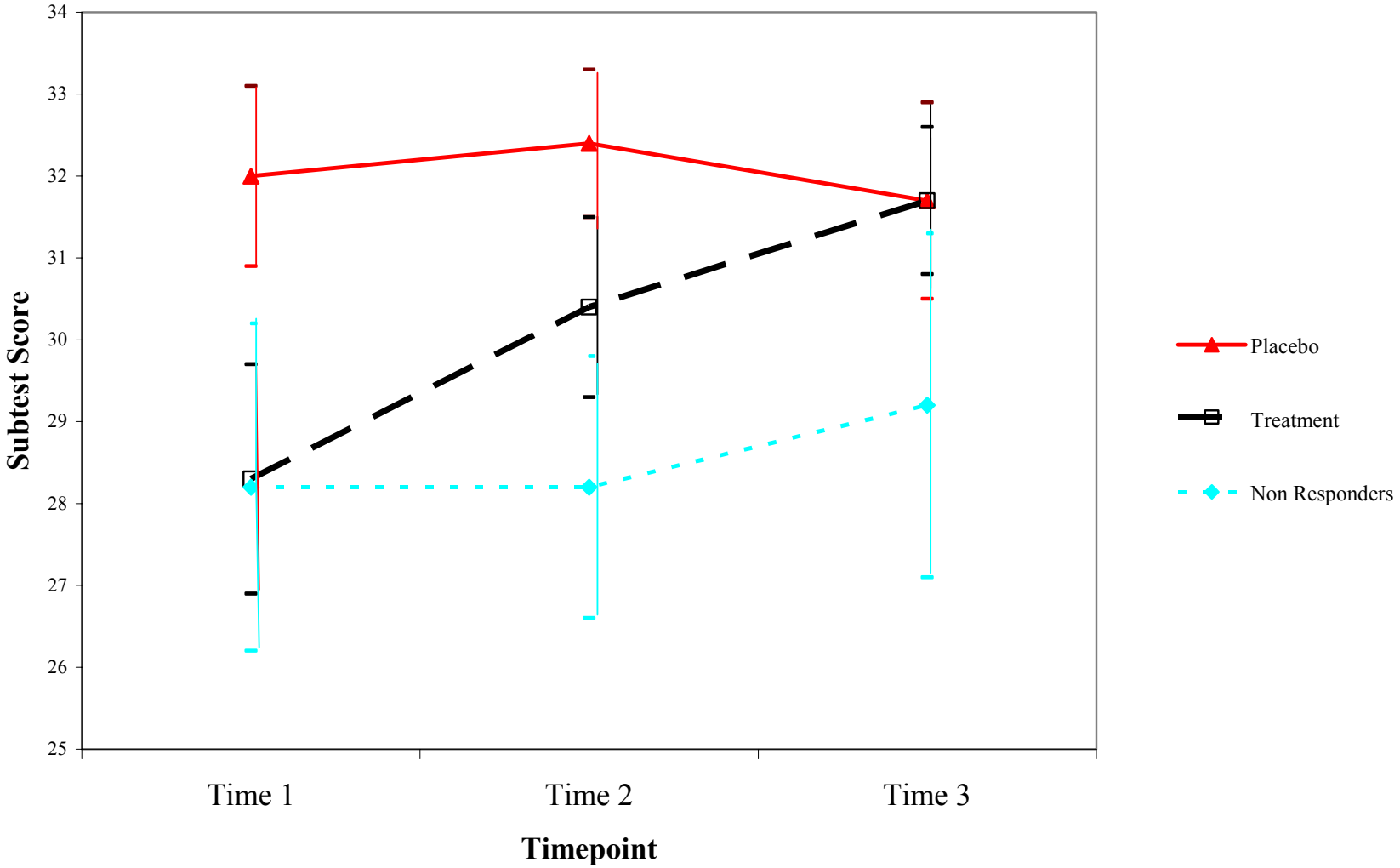


FIGURE 4.1: CSI Incidental Learning Index Scores across Time
Error bars represent standard error of the mean

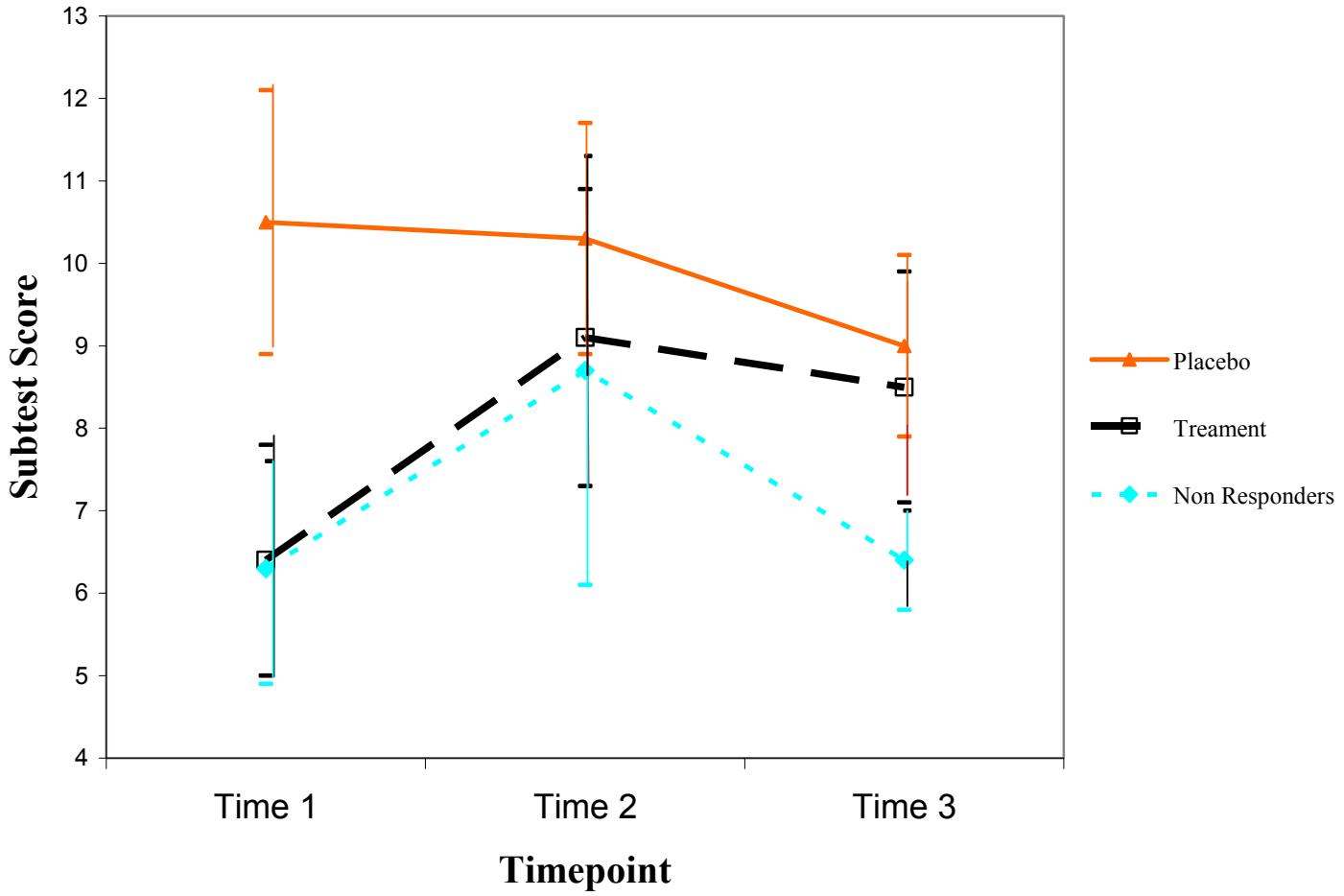


FIGURE 4.2: Matrix Reasoning Subtest Scores across Time
Error bars represent standard error of the mean

CHAPTER 5

CONCLUSIONS

Elevations in MMA were found to be prevalent among older adults in the study population (29%) and tied to cognitive impairment in the domains of processing speed, reasoning, verbal fluency and memory. Such domains are associated with fast and accurate processing of novel information rather than use of preexisting knowledge structures. Observed deficits suggest that the frontal and temporal regions of the brain may be most sensitive to vitamin B_{12} deficiency. Thus, all patients undergoing cognitive evaluation, but especially those with suspected frontotemporal dementia, should be evaluated for vitamin B_{12} deficiency. More specifically, as demonstrated in the study, special attention should be given to the measurement of MMA. Accurate and prompt testing for elevations in MMA is of particular importance as data from the current study suggest that vitamin B_{12} supplementation in persons with high MMA may reverse some cognitive decrements.

The metabolite MMA appeared to be a prevalent and sensitive indicator of cellular vitamin B_{12} deficiency and a better predictor of cognitive impairment than measures of serum vitamin B_{12} or tHcy. Measurement of serum vitamin B_{12} alone accounted for little to none of the variance in cognitive performance among the older adults in the study. Whereas tHcy levels appeared sensitive to cognitive decrement, only a small percentage of persons in the current study demonstrated high tHcy levels (N=13). The low prevalence rate of tHcy elevation reduced its utility as an indicator of B_{12} deficiency and related cognitive decrement in this sample of older adults receiving meals fortified with folate. In contrast, high MMA levels were prevalent in the

current study and related to unique variance in observed cognitive deficits. See Appendix A for statistical presentation of these relationships. Regression analyses demonstrated that MMA accounted for variance over and above that due to other biochemical variables (i.e., creatinine, serum B_{12}) in the areas of memory, cognitive speed and reasoning. Furthermore, MMA accounted for unique variance over and above that due to both biochemical and demographic variables (i.e., age, race, education, gender) in the reasoning ability of participants. On the basis of such findings, it can be alleged that persons with current high MMA levels are at increased risk of accelerated cognitive decline.

This raises the question of whether interventions designed to lower plasma MMA concentrations will prevent or reverse cognitive decline in older adults. The current study supports this possibility. Following a twelve month supplementation of vitamin B_{12}, those persons with high levels of MMA at baseline showed metabolic and cognitive improvements. Their cognitive ability increased on tasks of incidental learning and matrix reasoning. In fact, following vitamin B_{12} supplementation in participants with high MMA at baseline, performance on the matrix reasoning and incidental learning tasks rose to be at a level nearly identical to those without high MMA levels at baseline. Further research is needed to understand why participants did not show improvement in additional domains, including verbal fluency and processing speed. Additional research is also warranted to identify how MMA exerts its effects on cognition. The metabolic defect responsible for the cognitive decrements associated with MMA elevations remains unclear, as does the mechanism through which B_{12} supplementation reverses cognitive deficits.

Findings from the study delineating relationships between high MMA, vitamin B_{12} supplementation and cognitive performance in older adults are of enhanced value compared to

previous studies due to the use of well-validated and reliable neuropsychological measures in measuring cognition. Additionally, the period of twelve months, one of the longest to date, allowed for slow neuronal and subsequent cognitive recovery following vitamin B_{12} supplementation in persons with high MMA levels. Future studies of this sort may be improved through collection of information regarding length of B_{12} and cognitive dysregulation, as it is possible that irreversible damage may occur if B_{12} deficiency persists for long intervals.

Additionally, given that some, but not all, cognitive deficits were shown to be reversible with high dose oral B_{12} supplementation in persons with high MMA, additional research is needed to determine the amount of B_{12} needed to treat high MMA and the amount of B_{12} supplementation needed to prevent cognitive deficits. This may be accomplished through ensuring nutritional adequacy of vitamin B_{12} in older adults through fortification of food supply and/or appropriate use of dietary supplements in later life. Such measures may prove to be cost-effective through the prevention of elevated MMA in older adults. Through such interventions, frontal and temporal cognitive deficits may be prevented, in turn ultimately reducing functional impairments, emotional distress and dementia in later life.

APPENDICES

APPENDIX A
RELATIONSHIPS BETWEEN B_{12} INDICES (SERUM B_{12}, MMA, tHcy) AND COGNITIVE FUNCTION

1. Relationship between B_{12} deficiency as defined by Strict Definition (serum B_{12} <257 pmol/L, MMA > 271nmol/L & MMA > methylcitric acid) and Cognitive Function

CSI Factor:	B_{12}	N	Mean	s.d.	p	p**	*d*
Memory	+	96	5.6	1.7	NS	NS	.12
	-	16	5.4	1.5			
Info Proc Sp	+	95	-11.0	6.1	NS	NS	.32
	-	16	-14.0	12.9			
Reaction Time	+	89	-0.93	0.12	NS	NS	.42
	-	16	-1.00	0.21			
Attention	+	87	5.4	3.5	NS	NS	.02
	-	15	5.3	2.5			
COWAT	+	99	21.2	10.1	<.05	<.05	.68
	-	17	15.9	6.1			
Matrix Reasoning	+	99	9.1	6.4	NS	NS	.47
	-	17	6.4	5.0			

p* corrected for creatinine

2. Relationship between B_{12} deficiency as defined by High MMA (serum MMA >271 nmol/L) and Cognitive Function

CSI Factor:	MMA	N	Mean	s.d.	p	p*	p**	*d*
Memory	-	79	5.8	1.7	<.05	<.05	.057	.48
	+	33	5.0	1.6				
Info Proc Sp	-	78	-10.3	6.0	<.05	<.05	<.05	.47
	+	33	-14.0	9.8				
Reaction Time	-	74	-0.93	0.17	NS	NS	NS	.38
	+	31	-1.00	0.20				
Attention	-	72	5.7	3.4	NS	NS	NS	.31
	+	17	4.7	3.1				
COWAT	-	82	22.1	10.2	<.05	<.05	NS	.48
	+	34	17.8	7.9				
Matrix Reasoning	-	82	9.9	7.0	<.01	<.01	<.01	.69
	+	34	5.7	5.1				

p* corrected for creatinine
p** corrected for age, race, creatinine

3. Relationship between B_{12} deficiency as defined by High tHcy (serum tHcy >13.9 μmol/L) and Cognitive Function

CSI Factor:	B12	N	Mean	s.d.	p	p*	*d*
Memory	-	99	5.6	1.6	NS	NS	.27
	+	13	5.1	2.1			
Info Proc Sp	-	98	-11.0	7.4	NS	NS	.76
	+	13	-14.0	7.7			
Reaction Time	-	92	-0.94	0.17	NS	NS	.05
	+	13	-0.95	0.22			
Attention	-	89	5.5	3.4	NS	NS	.25
	+	13	4.7	3.1			
COWAT	-	103	21.7	10.0	<.05	.061	1.05
	+	13	14.3	4.1			
Matrix Reasoning	-	103	9.2	7.0	<.05	.072	.96
	+	13	4.4	3.0			

p* corrected for creatinine

4. Relationship between B_{12} deficiency as defined by Low serum B_{12} (serum B_{12} <257 pmol/L) and Cognitive Function

CSI Factor:	B12	N	Mean	s.d.	p	*d*
Memory	+	80	5.5	1.7	NS	.19
	-	32	5.8	1.5		
Info Proc Sp	+	79	-11.3	6.1	NS	.02
	-	32	-11.5	10.3		
Reaction Time	+	74	-0.94	0.15	NS	.05
	-	31	-0.95	0.23		
Attention	+	72	5.5	3.6	NS	.13
	-	30	5.1	2.8		
COWAT	+	83	21.5	9.4	NS	.22
	-	33	19.3	10.5		
Matrix Reasoning	+	83	8.7	7.0	NS	.02
	-	33	8.6	6.2		

APPENDIX B
NUTRITION, HEARING AND MEMORY AMONG SENIOR CENTER IN NORTHEAST GEORGIA CONSENT FORM AND TEST BOOK

NUTRITION, HEARING, AND MEMORY AMONG SENIOR CENTERS IN NORTHEAST GEORGIA

2001

*ID:*_____

FORM B

NUTRITION, HEARING, AND MEMORY STUDY
CONSENT FORM

I,_____agree to participate in the research titled "NUTRITION, HEARING, AND MEMORY" conducted by Drs. Mary Ann Johnson, Albert DeChicchis, and L. Stephen Miller in the Departments of Foods and Nutrition, Communication Sciences and Disorders, and Psychology at the University of Georgia.

I understand that I do not have to take part if I do not want to. I can stop taking part without giving any reason, and without penalty. I can ask to have all of the information about me returned to me, removed from the research records, or destroyed. My decision to participate will not effect the services that I receive at the Senior Center.

The reason for this study is to learn more about nutrition and health, and to determine if taking a vitamin B-12 supplement will help me hear better and improve my memory. If I volunteer to take part in this study, I will be asked to do the following things:

1) **Answer questions about my food, nutrition, and health.**
2) **Have my hearing tested.**
3) **Have my memory and thinking tested with a computer based test.**
4) **A medical technologist will take 4 7-10 ml tubes of blood to measure my blood sugar, cholesterol, vitamins and minerals. My blood sample will be destroyed within 10 years.**
5) **Have my blood pressure taken.**
6) **I will take a vitamin B-12 supplement (up to 1,000 mcg/day) or a placebo (a pill without vitamin B-12) for 4 months to see if it helps me hear and think better.**
7) **After 4 months, all of the questions and tests related to health, food, nutrition, hearing, and memory, and the blood tests will be repeated.**
8) **If my tests show that I have depression, I will be notified and referred for treatment.**
9) **Someone from the study may call me to clarify my information.**

If I am found to have vitamin B-12 deficiency, my physician and I will be notified. I will given vitamin B-12 (1,000 mcg/day as a tablet) as part of this study. If my doctor treats me with vitamin B-12 (pill or shots) I can still continue in this study, and will not need to take the vitamin B-12 supplement provided by this study.

I will receive $25 after completing all the test the first time, and another $25 after taking vitamin B-12 (or the placebo) for 4 months and repeating the tests a second time.

My blood will not be tested for HIV-AIDS. I understand that these questions and blood tests are not for diagnostic purposes. If I have questions about my test results I should see a physician. The benefits for me are that the study may help me understand and improve my health.

No risk is expected but I may experience some discomfort or stress when my hearing is tested (because of the ear plugs), when my blood is drawn or when the researchers ask me questions about my health, memory and nutrition. The risks of drawing blood from my arm include the unlikely possibilities of a small bruise or localized infection, bleeding, and fainting. These risks will be reduced in the following ways: my blood will be drawn only by a qualified and experienced person who will follow standard sterile techniques, who will observe me after the needle is withdrawn, and who will apply pressure to the blood draw-site. In the event that I have any health problems associated with the blood draws, my insurance or I will be responsible for any related medical expenses.

No information about me, or provided by me during the research, will be shared with others without my written permission, except if it is necessary to protect my welfare (for example, if I need physician care) or if required by law. I will be assigned an identifying number and this number will be used on all of forms I fill out.

If I have any further questions about the study, now or during the course of the project I can call Mrs. Nikki Hawthorne 706-542-4838 or Dr. Mary Ann Johnson 706-542-2292.

I give my permission for you to release my blood analysis information to my health care providers.
Circle one: YES / NO. Initial _____.

I give my permission for you to release my hearing results to my health care providers.
Circle one: YES / NO. Initial _____.

I give my permission for you to release my memory test results to my primary physician.
Circle one: YES / NO. Initial _____.

I will allow the staff to take my picture, videotape or record me while participating in the study. I can verbally refuse at anytime and my wishes will be upheld. My pictures will only be used to promote this nutrition, hearing, and memory study.
Circle one: YES / NO. Initial _____.
I understand that I am agreeing by my signature on this form to take part in this project and understand that I will receive a signed copy of this consent form for my records.

_____

Project Coordinator Date Signature of Participant Date

_____

Phone Number Address

Questions or problems regarding your rights as a participant should be addressed to Ms. Julia Alexander; Institutional Review Board; Office of V.P. for Research; The University of Georgia; 604A Graduate Studies Research Center; Athens, GA 30602-7411; Telephone 706-542-6514.

revised 00/11/29

UGA project number:H1998-10501-4
DHR project number: 000904

Vitamin B-12 Study Checklist
ID:_____

Questionnaire	PRE TEST		POST TEST		Flagged-Explain
	Date Completed	Initials	Date Completed	Initials	
Consent Form					
Blood Drawn					
General Information					
Sun Exposure					
Blood Pressure (Gave Blood Pressure Form to participant)					
Orientation/Memory Test					
Nutritional Screening Initiative					
MNA					
Nutrition Questions					
Illnesses					
Medications					
Supplements: Explained & Date Started					
Supplements: Stopped					
Nutritional Status Report - Sent to Individual					
Hearing History Questionnaire (HHQ)			**(E) ONLY**		
Hearing Handicap Inventory for adults (HHIA)					
Noise Exposure History					
Hearing Evaluation					
Cognition/computer-prompted test					
Geriatric Depression Scale					
Irritability and					

Agitation questions					

7
Flagged Notes:

_____

_____

_____

_____

_____

Revised 00/09/13

GENERAL INFORMATION ID: _____ (1-3)

_ _ _ _ _ _ (10-15) **1. Today's date: ___ / ____ / ____** ***Month/Day/Year***

__ (16) **2. This information was obtained from:**

0 _____ Client
1 _____ Senior center staff person
2 _____ Family member of client
3 _____ Caregiver for client
4 _____ Other: _____

__ __ . __ (17-20) **3. How long has the client been using the services of the senior center?**

___ ___.___ years *Code as years (xx.x years)*

_ _ _ _ _ _ _ _ (21-28) **4. Date of birth: ___ / ____ / ____** ***Month/Day/Year***

__ __ __ (29-31) **5. Current age: _____** years *Example: age 75 is 075*

__ (32) **6. Gender:** _____ Male (0) _____ Female (1)

__ (33) **7. Ethnicity:** _____ Caucasian (0) _____ Black (1) _____ Hispanic (2) _____ Asian (3) _____ Other (4)

__ __ (34-35) **8. Years completed in school?** _____ Years

__ (36) **9. Do you take a multiple-vitamin/mineral supplement?** _____ No (0) ____ Yes (1)

__ (37) **10. Do you take any other nutritional supplements that contain vitamins or minerals?**

_____ No (0) _____ Yes (1)

*** Health Care Provider** _____

Address_____

_____

Phone_____

*** Care giver/ Next of Kin**

(1) _____ **Phone**_____

Address_____

_____

(2) _____ **Phone**_____

Address_____

__ __
38-39

11. How many hours ago did you last eat? _____ (code number of hours ago).

__
40

12. Fasting status (coded by medical technologist).

0 Not fasted, food in the last 4 hours
1 Fasted, food not eaten in the past 4 hours

__
41

13. How would you rate your overall health at the present time -- excellent, good, fair, or poor?

3 Excellent
2 Good
1 Fair
0 Poor
9 Not answered

__
42

14. Is your health now better, about the same, or worse than it was five years ago?

2 Better
1 About the same
0 Worse
9 Not answered

__
43

15. How much do your health troubles stand in the way of your doing things you want to -- not at all, a little (some), or a great deal?

2 Not at all
1 A little (some)
0 A great deal
9 Not answered

__ __
44-45

16. County of residence **00-12**

00= Madison	03= Jackson	06= Greene	09= Elbert	12=Franklin
01= Morgan	04= Newton	07= Clark	10= Oconee	
02= Walton	05= Barrow	08= Oglethorpe	11= Jasper	

__
1999?
46

18. Did you participate in our vitamin supplement study during spring and summer

(ASK ONLY IN GREENE AND MORGAN COUNTY)

1= YES
0= NO

BLOOD PRESSURE

(NOTE: RECORD RESULTS ON "BLOOD PRESSURE FORM" AND GIVE TO PARTICIPANT)

__ __ __
50-52 **22. Blood Pressure**

Systolic (mmHg)

__ (0) < 120 Optimal
__ (1) < 130 Normal
__ (2) 130-139 High-normal
__ (3) 140-159 Mild Hypertension (Stage 1)
__ (4) 160-179 Moderate Hypertension (Stage 2)
__ (5) > 180 Severe Hypertension (Stage 3)
__ (999) Missing

Diastolic (mmHg)

__ __ __
53-55

__ (0) < 80 Optimal
__ (1) < 85 Normal
__ (2) 85-95 High-normal
__ (3) 90-99 Mild Hypertension (Stage 1)
__ (4) 100-109 Moderate Hypertension (Stage 2)
__ (5) > 110 Severe Hypertension (Stage 3)
__ (999) Missing

ORIENTATION-MEMORY-CONCENTRATION TEST

Read all questions to the participant. Tell them that some of the questions may be easy and some may be hard -- just do the best you can.

	Response	# of Errors	Max. Errors	Weight Factor	Total
1) What is the year now?			**1**	**4**	
2) What month is it now?			**1**	**3**	
Please repeat this phrase after me: **JOHN BROWN, 42 MARKET STREET, CHICAGO** *No score for this -- it is a memory phrase for Item # 6. Allow the person up to three trials for learning (repeating) the phrase. If the subject has not learned the phrase after three trials, record the value of "0" as the total score for Item #6, and proceed to Item #3.*					
3) Without looking at your watch or a clock, tell me about what time is it? *Note: score is correct if within one hour of actual time.*			**1**	**3**	
4) Count backwards from 20 to 1. *20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1*			**2**	**2**	
5) Say the months of the year in reverse order. *DEC, NOV, OCT, SEPT, AUG, JULY, JUNE, MAY, APR, MAR, FEB, JAN*			**2**	**2**	
6) Please repeat the name and address I asked you to remember. *Count the number of items (5) in memory phrase recalled incorrectly. An answer of either Market or Market Street is acceptable.* John / Brown / 42 / Market Street / Chicago			**5**	**2**	
(10-11) **TOTAL SCORE**					

Interpretation of corrected scores:

≤ 8	Normal or minimal impairment
9-19	Moderate impairment
≥ 20	Severe impairment

Source: Katzman, R., Brown, T., Fuld, P., Peck, A., Schechter, R., Schimmel, H. Validation of a short orientation-memory-concentration test of cognitive impairment. *American Journal of Psychiatry* 140: 734-739, 1983.

NUTRITIONAL HEALTH: Nutritional Screening Initiative Questionnaire

ID: _____

No=0
Yes=

____ (10) NH1. I have an illness or condition that made me change the kind and/or amount of food I eat.....Yes / No (2)

____ (11) NH2. I eat fewer than 2 meals per day.....Yes / No (3)

____ (12) NH3. I eat few fruits or vegetables, or milk products.....Yes / No (2)

____ (13) NH4. I have 3 or more drinks of beer, liquor or wine almost every day.....Yes / No (2)

____ (14) NH5. I have tooth or mouth problems that make it hard for me to eat .....Yes / No (2)

____ (15) NH6. I don't' always have enough money to buy the food I need.....Yes / No (4)

____ (16) NH7. I eat alone most of the time.....Yes / No (1)

____ (17) NH8. I take 3 or more different prescribed or over-the-counter drugs a day....Yes / No (1)

____ (18) NH9. Without wanting to, I have lost or gained 10 pounds in the last 6 months.....Yes / No (2)

____ (19) NH10. I am not always physically able to shop, cook, and/or feed myself.....Yes/No (2)

Your Nutritional Score is: _____. If it's:
(20-21)

0-2 **Good.** Recheck your nutritional score in 6 months.

3-5 **You are at moderate nutritional risk.** See what can be done to improve your eating habits and lifestyle. Your office on aging, senior nutrition program, senior citizens center or health department can help. Recheck your nutritional score in 3 months.

6 or more. You are at high nutritional risk. Bring this checklist the next time you see your doctor, dietitian or other qualified health or social service professional. Talk with them about any problems you may have. Ask for help to improve

MINI-NUTRITIONAL ASSESSMENT

ID# _____ (1-3)

Name: First name: Sex: Date:
Age:

04 (4-5) ** *enter decimal points*

TSF. Triceps skin fold (mm): __ __.__ (6-9)

Kneeht. Knee Height (cm): __ __.__ (10-13)

I. ANTHROPOMETRIC ASSESSMENT

____ MNA1. BMI (weight/(height)^2 in kg/m^2); weight = _____ lbs. / 2.205 = __ __ __ . __kg (14)
0 = BMI < 19
1 = 19 ≤ BMI < 21
2 = 21 ≤ BMI < 23
3 = BMI ≥ 23
height = _____ in. * .0254 = __ __ __ . __ meters
BMI = __ __ . __

(15-19)kg
(20-24)m
(25-28) BMI

_____ MNA2. Mid arm circumference (MAC in cm.): __ __ . __ cm. (29)
0.0 = MAC < 21
0.5 = 21 ≤ MAC ≤ 22
1.0 = MAC > 22

(30-33)cm

_____ MNA3. Calf circumference (CC in cm.): __ __ . __cm. (34)
0 = CC < 31
1 = CC ≥ 31

(35-38)cm

_____ MNA4. Weight loss during last 3 months: _____ lbs. / 2.205 = __ __ __ . __ kg (39) (40-44)kg
0 = weight loss > 3 kg
1 = does not know
2 = weight loss between 1 and 3 kg
3 = no weight loss

_____ MNA12A. How many servings of milk, yogurt, or cheese does the individual consume? (45)

0 ____ Less than one per week
1 ____ 1 per week
2 ____ 2 per week
3 ____ 3 per week
4 ____ 4 per week
5 ____ 5 per week
6 ____ 6 per week
7 ____ At least one per day
8 ____ 2 or more per day
9 ____ Missing/don't know

_____ MNA12D. How many servings of meat, fish, or poultry does the individual consume? (46)

0 ____ Less than one per week
1 ____ 1 per week
2 ____ 2 per week
3 ____ 3 per week
4 ____ 4 per week
5 ____ 5 per week
6 ____ 6 per week
7 ____ At least one per day
8 ____ 2 or more per day
9 ____ Missing/don't know

NUTRITION QUESTIONS

__ 47 **1. Have you ever received home delivered meals?**
0 = Yes
1 = No

__ __ 48-49 **2. If you receive home delivered meals, for how long have you been receiving them? _____ *years***
Code as whole years (xx years)

__ 50 **3. How many times a week do you eat at the senior center?**

0 ____ Less than one per week
1 ____ 1 per week
2 ____ 2 per week
3 ____ 3 per week
4 ____ 4 per week
5 ____ 5 per week
6 ____ 6 per week
7 ____ At least one per day
8 ____ 2 or more per day
9 ____ Missing/don't know

__ 51 **4. How many servings of green vegetables do you eat?**
0 ____ Less than one per week
1 ____ 1 per week
2 ____ 2 per week
3 ____ 3 per week
4 ____ 4 per week
5 ____ 5 per week
6 ____ 6 per week
7 ____ At least one per day
8 ____ 2 or more per day
9 ____ Missing/don't know

__ 52 **5. How many servings of orange or yellow vegetable do you eat?**
0 ____ Less than one per week
1 ____ 1 per week
2 ____ 2 per week
3 ____ 3 per week
4 ____ 4 per week
5 ____ 5 per week
6 ____ 6 per week
7 ____ At least one per day
8 ____ 2 or more per day
9 ____ Missing/don't know

__ 53 **6. How many servings of citrus fruit or citrus juice do you eat (e.g., orange, grapefruit)?**
0 ____ Less than one per week
1 ____ 1 per week
2 ____ 2 per week
3 ____ 3 per week
4 ____ 4 per week
5 ____ 5 per week
6 ____ 6 per week
7 ____ At least one per day
8 ____ 2 or more per day
9 ____ Missing/don't know

__ 54 **7. How many servings of other non-citrus fruit or juice do you consume?**
0 ____ Less than one per week
1 ____ 1 per week
2 ____ 2 per week
3 ____ 3 per week
4 ____ 4 per week
5 ____ 5 per week
6 ____ 6 per week
7 ____ At least one per day
8 ____ 2 or more per day
9 ____ Missing/don't know

__ 55 **8. How many servings of liver (eg., beef, chicken,pork) do you consume?**
0 ____ Less than one per week
1 ____ 1 per week
2 ____ 2 per week
3 ____ 3 per week
4 ____ 4 per week
5 ____ 5 per week
6 ____ 6 per week
7 ____ At least one per day
8 ____ 2 or more per day
9 ____ Missing/don't know

FOODS FORTIFIED WITH B-VITAMINS

We would like to know if you eat any of the following foods that may be fortified with B-vitamins

				Code daily intake of vit. B12 from each source.	Code daily intake of folate from each source.	Code daily intake of vit. B6 from each source.
8. Breakfast cereals, such as, Just Right w/ fruits & nuts, Product 19, Nutri-Grain, Total, Special K	**0 = No 1 = Yes**	**If yes, what BRAND(s) do you usually eat?**	**If yes, how often do you eat breakfast cereal?**			
9. Breakfast or energy bars, such as, Nutri-Grain, power bar,	**0 = No 1 = Yes**	**If yes, what BRAND(s) do you usually eat?**	**If yes, how often do you eat breakfast bars?**			
10. Liquid meal replacements, such as, carnation, ensure plus	**0 = No 1 = Yes**	**If yes, what BRAND(s) do you usually drink?**	**If yes, how often do you drink ensure, or boost etc.?**			
Other	**0 = No 1 = Yes**	**If yes, what BRAND(s) do you usually eat?**	**If yes, how often do you eat this food?**			
Other	**0 = No 1 = Yes**	**If yes, what BRAND(s) do you usually eat?**	**If yes, how often do you eat this food and in what quantity?**			

Are You at Risk for Osteoporosis?

Complete the following questionnaire to find out your risk for developing osteoporosis.

Question	Yes	No
1. Are you a postmenopausal women?		
2. If you are a postmenopausal woman, did you have an early (before 50 years old) menopause or surgically induced menopause?		
3. If you are a postmenopausal women, are you taking Hormone Replacement Therapy such as Raloxifene, Draloxifene, Premarin, Prempo?		
4. Do you have a small, thin frame?		
5. Has anyone in your family (father, mother, sister, brother) ever had a fracture or broken bone after age 50?		
6. Have you had a fall within the past 1 year?		
7. Have you had a fracture or broken bone after age 50?		
8. Do you eat at least 2 servings of dairy products such as milk, yogurt, or cheese everyday?		
9. Do you eat salmon at least twice a week?		
10. Do you eat calcium-rich green vegetables such as mustard, turnip, or collard greens everyday?		
11. Do you drink calcium-fortified juice everyday?		
12. Do you eat calcium-fortified cereals (such as Total, Kellogg's K) everyday?		
13. Do you take a calcium and vitamin D supplement everyday?		
14. Have you been taking excessive thyroid medication or high or prolonged doses of cortisone-like drugs for asthma, arthritis, or cancer?		
15. Do you currently or did you ever smoke cigarettes, pipes, cigars or chew tobacco on a daily basis?		
16. Do you exercise at least 30 minutes everyday?		

(NOTE: COPY THIS INFORMATION FROM PREVIOUS QUESTIONS)

17. Age : _____ years old
18. Gender : Male Female (please circle)
19. Ethnicity : White Black Hispanic Asian Others (please circle)
20. County : _____
21. Height : _____ feet _____ inches OR _____ cm
22. Weight : _____ pounds OR _____ kg

Is height and weight measured or self-reported?

The more times you answer in the shaded boxes, the greater your risk for developing osteoporosis. See your physician.

MEDICATIONS AND ILLNESSES

NAME/ID: _____

Obtain information from reliable source. This information was provided by: client, caretaker, other ?

	YES (1)	NO (0)	DON'T KNOW	Space
Total number of PRESCRIPTION medications				10-11
Total number of NON -PRESCRIPTION medications, not counting vitamins and minerals				12-13
Multiple vitamin mineral supplement? 0 = no, 1 = yes				14
Number of other nutritional supplements?				15
Total number of illnesses - fill in when finished below.				16-17
1) Anemia in the past year				18
2) Alzheimer's: Kind_____; Dx date_____				19
3) Other dementias: Kind_____; Dx date _____				20
4) Cancer: Kind_____; Dx date_____; Status_____				21
5) Circulatory problems in the past year				22
6) Congestive heart failure in the past year				23
7) Constipation in the past year				24
8) Diabetes: Kind_____; Dx date _____				25
9) Diarrhea in the past year				26
10) Glaucoma in the past year				27
11) Hearing problems in the past year				28
12) Heart disease in the past year				29
13) Hypertension in the past year				30
14) Legally blind in the past year				31
15) Liver disease in the past year				32
16) Mental illness: Kind_____; Dx date_____				33
17) Osteoporosis in the past year				34
18) Hip fracture in the past year				35
19) Have you every had a pace maker				36
20) Parkinson's disease: Dx date_____				37
21) Renal disease in the past year				38
22) Respiratory disease in the past year				39
23) Seizures: 1st date_____; last date_____				40
24) Skin rashes, bed sores in the past year				41
25) Stroke: Number_____; Dates_____				42
26) Thyroid problems: Kind_____; Dx date_____				43
27) Visual disturbances in the past year				44
28) Cataracts in the past year				45
29) Have you used any type of tobacco in the past year				46
30) Have you every had stomach surgery				47
31) Emergency room visit in the past year				48
32) Other				49
33) Arthritis in the past year				50
34) Pneumonia in the past year				51
35) Dizziness in the past year				52
36) Gout in the past year				53
37)				54

MEDICATIONS ***(NOTE: ASK EVERY MEDICATION QUESTION THEN RECORD MEDS ON THE NEXT FORM)***			
1)	Are you currently taking aspirin?	1 = Yes 0 = No	10
2)	Are you currently taking ibuprofen such as Advil, Motrin, Nuprin?	1 = Yes 0 = No	11
3)	Are you currently taking Aleve?	1 = Yes 0 = No	12
4)	Are you currently taking Acetaminophen such as Tylenol or similar medication?	1 = Yes 0 = No	13
5)	Are you currently taking antacids or medications for heartburn or indigestion such as maalox, mylanta, alka aid (alka-seltzer) gaviscon, propulsid, zantac, pepcid, axid, cyotec, tums, tagamet, proton pump inhibitors such as prevacid, prevapac,prilosec, or other medication? **CIRCLE ALL THAT APPLY**	1 = Yes 0 = No	14
6)	Are you currently taking laxatives such as milk of magnesia, fiber tablets, metamucil or other laxative medication? **CIRCLE ALL THAT APPLY**	1 = Yes 0 = No	15
7)	Are you currently taking a cough suppressant such as humibid, robitussin, entrex or other medication?	1 = Yes 0 = No	16
8)	Are you currently taking allergy, sinus, or cold medication such as chlorpheniramine, relief, allerfed, seldane, sudafed, sine aid, Tylenol allergy sinus, contac, tylenol cold formulas, methypred dose, claritin, phenylprop, guaif, bromfed, tavist-d, actifed, benadryl, equate allergy sinus or other medication?	1 = Yes 0 = No	17
9)	Are you currently using nasal spray for allergy or sinus, such as aerobid, flonase, beconase, Nasalcrom or other medication?	1 = Yes 0 = No	18
10)	Are you currently taking a non-steroidal anti-inflammatory drug (NSAID) such as voltaren, diclofenac, naprosyn, naproxyn, sulindac, lodine, relafen, daypro, oruvail or similar medication?	1 = Yes 0 = No	19
11)	Are you currently taking a pain medication such as ultram, darvocet-N-100, fiorinal or similar medication?	1 = Yes 0 = No	20
12)	Are you currently taking an arthritis medication such as prednisone, rheumatrex methotrexate, orasone, deltasone or other medication?	1 = Yes 0 = No	21
13)	Are you currently taking antibiotics such as zithromax, amoxicillin or other antibiotic medication?	1 = Yes 0 = No	22
14)	Are you currently taking a sleeping aid such as Tylenol PM or other medication?	1 = Yes 0 = No	23

MEDICATIONS			
15)	Are you currently taking migraine medication such as mepergan fortis, imitrex, ercaf, Forbal-S or other migraine medication?	1 = Yes 0 = No	24
	# 16 and #18 - important for cognitive tests - so probe carefully		
16)	Are you currently taking anti-anxiety medication such as Alprazolam (xanax), Buspirone (Buspar), Chlordiaxepoxide (Librium), Clonazepam (klonopin), Clorazepate (tranxene), Diazepam (Valium), Hydroxyzine (Vistaril), Lorazepam (Ativan), Oxazepam (Serax), Propanolol (Inderal) or other anti-anxiety medication? **Circle all that apply**	1 = Yes 0 = No	25
17)	Are you currently taking anti-depressant medication such as Amitriptyline (Elavil), Citalopram (Celexa), Clomipramine (Anafranil), Desipramine (Norpramin), Doxepin (Sinequan), Fluoxetine (Prozac), Fluvoxamine (Luvox), Imipramine (Tofranil), Maprotiline (Ludiomil), Nortriptyline (Pamelor), Paroxetine (Paxil), Sertraline (zoloft), Trazadone (Desyrel), Venlafaxine (Effexor) or other anti-depressant medication? **Circle all that apply**	1 = Yes 0 = No	26
	#41 & 42 - important for cognitive tests - so probe carefully		
41)	Are you currently taking any drugs to help or enhance your thinking such as Chlorpromazine (Thorazine), Thioridazine (Mellaril), Fluphenazine (Prolixin), Trifluoperazine (Stelazine), Haloperidol (Haldol), Thiothixene (Navane), Loxapine (Loxitane), Molindone (Moban), Clozapine (Clozaril), Risperidone (Risperdal), Quetiapine (Seroquel), Olanzapine (Zyprexa) or other neuroleptic medications? **Circle all that apply**	1 = Yes 0 = No	27
42)	Are you currently taking any drugs to help or enhance your memory such as Tacrine (Cognex) or Donepezil hydrochloride (Aricept)? **Circle all that apply**	1 = Yes 0 = No	28
43)	List any other medications currently taken:	1 = Yes 0 = No	29
45)	Are you currently receiving Vitamin B-12 injections/shots? Last Vitamin B-12 shot (date) : _____ How often? _____(*example: once a year, twice a year, every other month, once a month) 762* ***(NOTE: IF YES, THEY NEED TO HAVE HAD A SHOT 6 MONTHS AGO OR LATER AND AGREE NOT TO RECEIVE A SHOT FOR THE NEXT FOUR MONTHS TO PARTICIPATE IN THIS STUDY)***	1 = Yes 0 = No	30
	Total number of prescription medications *(total of prescription meds)*		31-32
	Total number of non-prescription medications *(total of nonprescription meds)*		33-34

RESIDENT MEDICATION RECORDS:
Include prescription, non-prescription, and vitamins/minerals

ID _____

Medication	Dosage	Schedule	Route of Administration	*How Long have you been taking medication	DOCUMENTATION (Check all that apply)		
					Bottle only	Cardex Record	Signature Administration Record

	_____ ***SUPP # __***	_____ ***SUPP # __***	_____ ***SUPP # __***	_____ ***SUPP # __***	_____ ***SUPP # __***	***TOTAL***
	__ # pills per D, W, M **WRITE IN AMOUNT /PILL & CIRCLE UNIT**	**__ # pills per D, W, M** **WRITE IN AMOUNT /PILL & CIRCLE UNIT**	**__ # pills per D, W, M** **WRITE IN AMOUNT /PILL & CIRCLE UNIT**	**__ # pills per D, W, M** **WRITE IN AMOUNT /PILL & CIRCLE UNIT**	**__ # pills per D, W, M** **WRITE IN AMOUNT /PILL & CIRCLE UNIT**	
For how long?	**_____mo/yrs**	**_____mo/yrs**	**_____mo/yrs**	**_____mo/yrs**	**_____mo/yrs**	
Vitamin A	IU RE	IU RE	IU RE	IU RE	IU RE	
Vitamin C	mg	mg	mg	mg	mg	
Vitamin D	IU mg	IU mg	IU mg	IU mg	IU mg	
Vitamin E	IU mg	IU mg	IU mg	IU mg	IU mg	
Thiamin (B1)	mg	mg	mg	mg	mg	

Riboflavin (B2)	mg	mg	mg	mg	mg	
Niacin or Niacinamide or Vit. B3	mg	mg	mg	mg	mg	
Pyridoxine or Vitamin B6	mg	mg	mg	mg	mg	
Folic acid or Folate	mcg mg	mcg mg	mcg mg	mcg mg	mcg mg	
Vitamin B-12	mg mcg	mg mcg	mg mcg	mg mcg	mg mcg	
Biotin	mg mcg	mg mcg	mg mcg	mg mcg	mg mcg	
Pantothenic Acid	mg	mg	mg	mg	mg	
Vitamin K	mcg	mcg	mcg	mcg	mcg	
Calcium	mg	mg	mg	mg	mg	
Iron	mg	mg	mg	mg	mg	
Phosphorus	mg	mg	mg	mg	mg	
Iodine	mcg	mcg	mcg	mcg	mcg	
Magnesium	mg	mg	mg	mg	mg	
Zinc	mg	mg	mg	mg	mg	
Copper	mg	mg	mg	mg	mg	
Potassium	mg	mg	mg	mg	mg	
Manganese	mg	mg	mg	mg	mg	
Chromium	mcg	mcg	mcg	mcg	mcg	
Molybdenum	mcg	mcg	mcg	mcg	mcg	
Chloride	mg	mg	mg	mg	mg	
Nickel	mcg	mcg	mcg	mcg	mcg	
Silicon	mg mcg	mg mcg	mg mcg	mg mcg	mg mcg	
Vanadium	mcg	mcg	mcg	mcg	mcg	
Boron	mg mcg	mg mcg	mg mcg	mg mcg	mg mcg	
Fluoride	mg	mg	mg	mg	mg	
Selenium	mcg	mcg	mcg	mcg	mcg	
Other						
	SUPP # __	***SUPP # __***	***SUPP # __***	***SUPP # __***	***SUPP # __***	***TOTAL***

	__ # pills per D, W, M WRITE IN AMOUNT /PILL & CIRCLE UNIT	__ # pills per D, W, M WRITE IN AMOUNT /PILL & CIRCLE UNIT	__ # pills per D, W, M WRITE IN AMOUNT /PILL & CIRCLE UNIT	__ # pills per D, W, M WRITE IN AMOUNT /PILL & CIRCLE UNIT	__ # pills per D, W, M WRITE IN AMOUNT /PILL & CIRCLE UNIT	
For how long?	**_____mo/yrs**	**_____mo/yrs**	**_____mo/yrs**	**_____mo/yrs**	**_____mo/yrs**	
Vitamin A	IU RE	IU RE	IU RE	IU RE	IU RE	
Vitamin C	mg	mg	mg	mg	mg	
Vitamin D	IU mg	IU mg	IU mg	IU mg	IU mg	
Vitamin E	IU mg	IU mg	IU mg	IU mg	IU mg	
Thiamin (B1)	mg	mg	mg	mg	mg	
Riboflavin (B2)	mg	mg	mg	mg	mg	
Niacin or Niacinamide or Vit. B3	mg	mg	mg	mg	mg	
Pyridoxine or Vitamin B6	mg	mg	mg	mg	mg	
Folic acid or Folate	mcg mg	mcg mg	mcg mg	mcg mg	mcg mg	
Vitamin B-12	mg mcg	mg mcg	mg mcg	mg mcg	mg mcg	
Biotin	mg mcg	mg mcg	mg mcg	mg mcg	mg mcg	
Pantothenic Acid	mg	mg	mg	mg	mg	
Vitamin K	mcg	mcg	mcg	mcg	mcg	
Calcium	mg	mg	mg	mg	mg	
Iron	mg	mg	mg	mg	mg	
Phosphorus	mg	mg	mg	mg	mg	
Iodine	mcg	mcg	mcg	mcg	mcg	
Magnesium	mg	mg	mg	mg	mg	
Zinc	mg	mg	mg	mg	mg	
Copper	mg	mg	mg	mg	mg	
Potassium	mg	mg	mg	mg	mg	
Manganese	mg	mg	mg	mg	mg	
Chromium	mcg	mcg	mcg	mcg	mcg	
Molybdenum	mcg	mcg	mcg	mcg	mcg	
Chloride	mg	mg	mg	mg	mg	
Nickel	mcg	mcg	mcg	mcg	mcg	
Silicon	mg mcg	mg mcg	mg mcg	mg mcg	mg mcg	
Vanadium	mcg	mcg	mcg	mcg	mcg	
Boron	mg mcg	mg mcg	mg mcg	mg mcg	mg mcg	
Fluoride	mg	mg	mg	mg	mg	
Selenium	mcg	mcg	mcg	mcg	mcg	

HEARING HANDICAP INVENTORY FOR ADULTS (HHIA)

Date: _____ ID: _____

The purpose of these questions is to identify any problems your hearing loss may be causing you. Please do not skip any questions. Even if you feel you do not have a hearing loss, please answer all of the questions. For each question, circle one response: No, Sometimes, or Yes.

		0	2	4	Line Space Line # 4-5
S1	Does a hearing problem cause you to use the phone less often than you would like?	No	Sometimes	Yes	10
E2*	Does a hearing problem cause you to feel embarrassed when meeting new people?	No	Sometimes	Yes	11
S3	Does a hearing problem cause you to avoid groups of people?	No	Sometimes	Yes	12
E4	Does a hearing problem make you irritable?	No	Sometimes	Yes	13
E5*	Does a hearing problem cause you to feel frustrated when talking to members of your family?	No	Sometimes	Yes	14
S6	Does a hearing problem cause you difficulty when attending a party?	No	Sometimes	Yes	15
S7	Does a hearing problem cause you difficulty hearing/understanding coworkers, clients, or customers?	No	Sometimes	Yes	16
E8*	Do you feel handicapped by a hearing problem?	No	Sometimes	Yes	17
S9*	Does a hearing problem cause you difficulty when visiting friends, relatives, or neighbors?	No	Sometimes	Yes	18
E10	Does a hearing problem cause you to feel frustrated when talking to coworkers, clients, or customers?	No	Sometimes	Yes	19
S11*	Does a hearing problem cause you difficulty in the movies or theater?	No	Sometimes	Yes	20
E12	Does a hearing problem cause you to be nervous?	No	Sometimes	Yes	21
S13	Does a hearing problem cause you to visit friends, relatives, or neighbors less often than you would like?	No	Sometimes	Yes	22
E14*	Does a hearing problem cause you to have arguments with family members?	No	Sometimes	Yes	23
S15	Does a hearing problem cause you difficulty when listening to the TV or radio?	No	Sometimes	Yes	24
E16	Does a hearing problem cause you to go shopping less often than you would like?	No	Sometimes	Yes	25
E17	Does any problem or difficulty with your hearing upset you at all?	No	Sometimes	Yes	26
E18	Does a hearing problem cause you to want to be by yourself?	No	Sometimes	Yes	27
S19	Does a hearing problem cause you to talk to family members less often than you would like?	No	Sometimes	Yes	28
E20*	Do you feel that any difficulty with your hearing limits or hampers your personal or social life?	No	Sometimes	Yes	29
S 21	Does a hearing problem cause you difficulty when	No	Sometimes	Yes	30

	in a restaurant with relatives or friends?				
E 22	Does a hearing problem cause you to feel depressed?	No	Sometimes	Yes	31
S 23	Does a hearing problem cause you to listen to TV or radio less often than you would like?	No	Sometimes	Yes	32
E 24	Does a hearing problem cause you to feel uncomfortable when talking to friends?	No	Sometimes	Yes	33
E 25	Does a hearing problem cause you to feel left out when you are with a group of people?	No	Sometimes	Yes	34
E 26	Does a hearing problem cause you to feel "stupid" or "dumb"?	No	Sometimes	Yes	35
S 27	Do you have difficulty hearing when someone speaks in a whisper?	No	Sometimes	Yes	36
S 28	Does a hearing problem cause you to attend religious services less often than you would like?	No	Sometimes	Yes	37

* Items comprising the HHIA-S.

From: Newman, C.W., Weinstein, B.E., Jacobson, G.P., and Hug, G.A. Test-retest reliability of the Hearing Handicap Inventory for Adults, Hearing and Hearing, 12(5): 355-

NOISE EXPOSURE HISTORY

We need to know about noise exposure in your past, even as a child. An example of a loud noise is a noise that makes it hard to talk or hear another person, or makes your ears ring after exposure.

NOISE AT YOUR WORK **1. Have you had any of these jobs?**			**Date Started**	**Date Ended**	**How often did you use hearing protection?** **Never Sometimes Always**		
A. Cannery	No	Yes	19_____	_____	1	2	3
B. Construction	No	Yes	19_____	_____	1	2	3
C. Factory: _____ *(type of factory)*	No	Yes	19_____	_____	1	2	3
D. Farming	No	Yes	19_____	_____	1	2	3
E. Logging, Lumber industry	No	Yes	19_____	_____	1	2	3
F. Loud music (performing)	No	Yes	19_____	_____	1	2	3
G. Mining	No	Yes	19_____	_____	1	2	3
H. Police, Fire, Dept.	No	Yes	19_____	_____	1	2	3
I. Printing	No	Yes	19_____	_____	1	2	3
J. Transportation *(truck, boat, plane...)*	No	Yes	19_____	_____	1	2	3
K. Any other types of noisy jobs Describe_____	No	Yes	19_____	_____	1	2	3

NOISE DURING MILITARY SERVICE **2. Were you exposed to noise during military service (including basic training and reserves)?**			**Date Started**	**Date Ended**	**How often did you use hearing protection?** **Never Sometimes Always**		
A. Artillery	No	Yes	19_____	_____	1	2	3
B. Explosion	No	Yes	19_____	_____	1	2	3
C. Planes, helicopters	No	Yes	19_____	_____	1	2	3
D. Small arms	No	Yes	19_____	_____	1	2	3
E. Tanks, other heavy equipment	No	Yes	19_____	_____	1	2	3
F. Other types of noise: Describe_____	No	Yes	19_____	_____	1	2	3

NOISE DURING RECREATION **3. Have you been exposed to noise during recreational or leisure-time activities?**		**Date Started**	**Date Ended**	**How often did you use hearing protection?** **Never**	**Sometimes**	**Always**
A. Gunfire	No	Yes	19_____	_____	1	2 3
B. Loud Engines *(boat, auto, plane, motorcycle, skimobile)*	No	Yes	19_____	_____	1	2 3
C. Loud Music	No	Yes	19_____	_____	1	2 3
D. Power Tools	No	Yes	19_____	_____	1	2 3
E. Other types of noise: Describe_____	No	Yes	19_____	_____	1	2 3

Have you ever undergone any accidental exposure to sudden intense noise?

No 1
Yes 2 Type of noise_____
Your age then_____

Which ear or side?
LEFT ear 1 BOTH ears 3
Right ear 2 Not sure 4

Adapted fom Meikle, Griest & Press (1986)

Geriatric Depression Scale (GDS) Short form

Choose the best answer for how you felt over the past week. Please answer the following questions "YES" or "NO there are no right or wrong answers, only what best applies to you.

		1 0	Space
1)	Are you basically satisfied with your life?	Yes *NO	10
2)	Have you dropped many of your activities and interests?	*YES No	11
3)	Do you feel that your life is empty?	*YES No	12
4)	Do you often get bored?	*YES No	13
5)	Are you in good spirits most of the time?	Yes *NO	14
6)	Are you afraid that something bad is going to happen to you?	*YES No	15
7)	Do you feel happy most of the time?	Yes *NO	16
8)	Do you often feel helpless?	*YES No	17
9)	Do you prefer to stay at home, rather than going out and doing new things?	*YES No	18
10)	Do you feel you have more problems with memory than most people?	*YES No	19
11)	Do you think it is wonderful to be alive now?	Yes *NO	20
12)	Do you feel pretty worthless the way you are now?	*YES No	21
13)	Do you feel full of energy?	Yes *NO	22
14)	Do you feel that your situation is hopeless?	*YES No	22
15)	Do you think that most people are better off than you are?	*YES No	23

*** = 1 point. If * score is 10 or greater, or if (Nos. 1,5,7,11,13) were answered with * then the participant may be depressed. Proceed with referral plan.**

In the last few weeks have you found things to be easily disturbing or annoying (e.g., have other people, objects or situations been getting on your nerves or causing you frustration?)

1 2 3 4 5 6 7

Not at all all of the time

In the last few weeks have you felt restless or experienced difficulty with activities such as sleeping, following instructions, keeping your mind on what you are doing?

1 2 3 4 5 6 7

Not at all all of the time

NUTRITION AND DEPRESSION STATUS REPORT

NAME: _____

COUNTY: _____

NUTRITION SCREENING INITIATIVE - 10 ITEM QUESTIONNAIRE:

This questionnaire screens for nutritional problems.

_____	0-2	Good
_____	3-5	Moderate nutritional risk
_____	6 or more.	High nutritional risk; recommend nutrition consult

BODY MASS INDEX (KG/M2) - INDEX OF WEIGHT FOR HEIGHT:

This is an index of underweight, normal weight, overweight and obesity.

_____ Greater than 30 Obese; recommend nutrition consult

_____ 25-29 Overweight; At risk for nutrition problems; recommend nutrition consult

_____ 21-24.9 Normal Range

_____ Less than 19.9; At risk for nutrition problems; recommend nutrition consult

WEIGHT LOSS (> 3 KG or 7 POUNDS IN PREVIOUS 3 MONTHS): _____

Unintentional weight loss is an indicator of low food intake or illness. However, some people need to lose weight if they are overweight and their weight is contributing to health problems.

_____ No weight loss Good

_____ Weight loss > 7 lb At risk for nutrition problems; recommend nutrition consult

PLEASE FEEL FREE TO CONTACT NIKKI HAWTHORNE TO MAKE AN APPOINTMENT FOR A NUTRITIONAL CONSULT: 706-542-4838

GERIATRIC DEPRESSION SCALE- 15 ITEM QUESTIONNAIRE:_____

This questionnaire measures depression.

_____ 9 or less; probably not depressed

_____ 10 or more; at risk for depression - contact senior center director

APPENDIX C
TIME 3 DATA COLLECTION CONSENT FORM

NUTRITION, HEARING, AND MEMORY STUDY CONSENT FORM

I,_____agree to participate in a continuation to the research titled "NUTRITION, HEARING, AND MEMORY" conducted by Drs. Mary Ann Johnson, Albert DeChicchis, and L. Stephen Miller in the Departments of Foods and Nutrition, Communication Sciences and Disorders, and Psychology at the University of Georgia.

I understand that I do not have to take part if I do not want to. I can stop taking part without giving any reason, and without penalty. I can ask to have all of the information about me returned to me, removed from the research records, or destroyed. My decision to participate will not effect the services that I receive at the Senior Center.

The reason for this continuation to this study is to learn more about nutrition and health, and to determine if taking a vitamin B-12 supplement will help improve my memory. If I volunteer to take part in this study, I will be asked to do the following things:

10) **Answer questions about my food, nutrition, and health.**
11) **Have my memory and thinking tested with a computer based test.**
12) **A medical technologist will take 2 7-10 ml tubes of blood to measure my vitamin B-12 status. My blood sample will be destroyed within 10 years.**
13) **Have my blood pressure taken.**
14) **I will take a vitamin B-12 supplement (up to 1,000 mcg/day) or a placebo (a pill without vitamin B-12) for 6 months to see if it helps me think better.**
15) **If my tests show that I have depression, I will be notified and referred for treatment.**
16) **Someone from the study may call me to clarify my information.**

I will receive $25 after completing all of the testing.
My blood will not be tested for HIV-AIDS. I understand that these questions and blood tests are not for diagnostic purposes. If I have questions about my test results I should see a physician. The benefits for me are that the study may help me understand and improve my health.

No risk is expected but I may experience some discomfort or stress when my blood is drawn or when the researchers ask me questions about my health, memory and nutrition. The risks of drawing blood from my arm include the unlikely possibilities of a small bruise or localized infection, bleeding, and fainting. These risks will be reduced in the following ways: my blood will be drawn only by a qualified and experienced person who will follow standard sterile techniques, who will observe me after the needle is withdrawn, and who will apply pressure to the blood draw-site. In the event that I have any health problems associated with the blood draws, my insurance or I will be responsible for any related medical expenses.

No information about me, or provided by me during the research, will be shared with others without my written permission, except if it is necessary to protect my welfare (for example, if I need physician care) or if required by law. I will be assigned an identifying number and this number will be used on all forms I fill out.

If I have any further questions about the study, now or during the course of the project I can call Mrs. Nikki Hawthorne 706-542-4838 or Dr. Mary Ann Johnson 706-542-2292.

I give my permission for you to release my blood analysis information to my health care providers.
Circle one: YES / NO. Initial _____.

I give my permission for you to release my memory test results to my primary physician.
Circle one: YES / NO. Initial _____.

I will allow the staff to take my picture, videotape or record me while participating in the study. I can verbally refuse at anytime and my wishes will be upheld. My pictures will only be used to promote this nutrition, hearing, and memory study.
Circle one: YES / NO. Initial _____.

I understand that I am agreeing by my signature on this form to take part in this project and understand that I will receive a signed copy of this consent form for my records.

_____

Project Coordinator Date Signature of Participant Date

_____

Phone Number Address

Questions or problems regarding your rights as a participant should be addressed to Dr. Chris Joseph; Institutional Review Board; Office of V.P. for Research; The University of Georgia; 604A Graduate Studies Research Center; Athens, GA 30602-7411; Telephone 706-542-6514.

UGA project number:H1998-10501-4
DHR project number: 000904

5/16/01

APPENDIX D
SELECTED SUBTESTS FROM THE COGNITIVE STABILITY INDEX

Headminder™ Subtests – Partial List

ATTENTION

Number Recall
A sequence of numbers is presented one at a time. Afterwards the subject must enter the numbers in the same order. Sequences begin with two digits and increase in span. Five alternate forms available.

Sequences of numbers appear, one number at a time, briefly on the screen.

Patients then enter that number sequence.

Sequences get longer until errors establish an upper limit of capacity.

Number Sequencing
A series of numbers is presented. Afterwards the subject must enter the numbers in numerical order. Sequences increase in span. Five alternate forms available.

*Sequences of numbers appear, one number at a time, briefly on the screen***.**

Patients then enter the numbers, in ascending order.

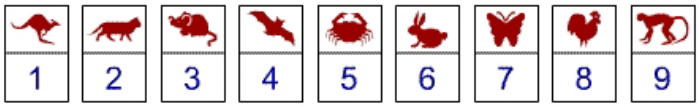
Sequences get longer until errors establish an upper limit of capacity.

PROCESSING SPEED

Animal Decoding

Based on a legend, individuals must enter, as quickly as possible, the appropriate number in each empty box. Five alternate forms are available.

A legend pairing animals with numbers is provided.



Animals are subsequently presented with empty boxes beneath. Patients enter the corresponding number into each empty box as quickly as possible.

Symbol Scanning

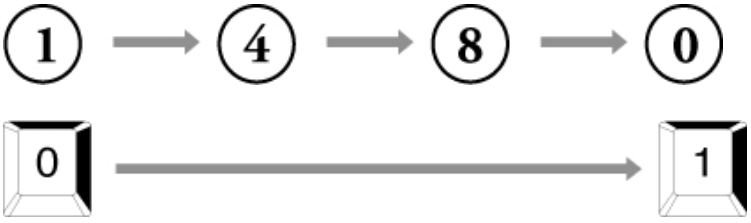
A pair of shapes appear to the left and a group of eight shapes appear to the right. Patients determine if either one or both shapes on the left appear on the right and answer either "1" or "2".

REACTION TIME

Response Direction 1

A series of numbers is displayed one at a time. Patients press the "1" key when the number 1 appears on the screen and the "0" key when the number 0 appears.

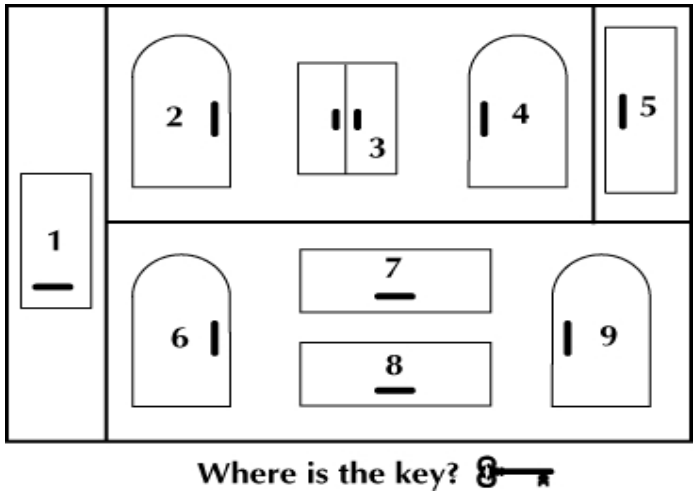
Response Direction 2
A series of numbers is displayed one at a time. Patients press the "1" key when the number 0 appears on the screen and the "0" key when the number 1 appears.



MEMORY

Memory Cabinet 1
Individuals learn the placement of 9 household objects placed in a cabinet over 3 trials. Alternate forms are randomly generated.

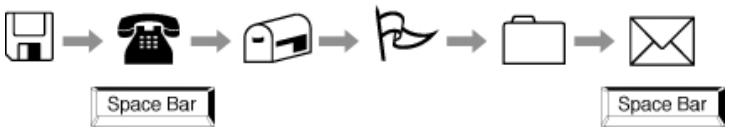
Memory Cabinet 2
Companion test to *Memory Cabinet 1*. Following approximately 10-minutes of intervening tasks, one recall trial of the Memory Cabinet is administered. No reminders are provided.



Incidental Learning 1
A series of pictures appears on the screen, one picture at a time. Individuals are instructed to press the spacebar as quickly as possible whenever they see a picture of any type of plant (flower, vegetable, fruit, etc.).

Incidental Learning 2

Companion test to *Incidental Learning 1.* Administered after an intervening task. Individuals are instructed to press the spacebar as quickly as possible whenever they recognize a picture from *Incidental Learning 1.*



APPENDIX E
THE CONTROLLED ORAL WORD ASSOCIATION TEST (COWAT)

"Now we're going to do something different. In a minute I'm going to ask you to tell me all the words you can think of that begin with a certain letter. There are some rules to this test. First, you cannot say the same word more than once. Second, you can not say various forms of the same word, so for example, if the letter were *p* and you said *paint* you could not also say *painting* and *painter*. Third, you can not use proper nouns. So, if the letter was *p*, you could say *paper* and *pretty*, but you could not say *Peter, Patty or Philadelphia.* Do you have any questions?"
"Okay, in one minute, tell me all the words you can think of that begin with the letter…"

C

_____ _____ _____

_____ _____ _____

_____ _____ _____

_____ _____ _____

Repeat above instructions for letter F.

F

_____ _____ _____

_____ _____ _____

_____ _____ _____

_____ _____ _____

Repeat above instructions for letter L.

L

_____ _____ _____

_____ _____ _____

_____ _____ _____

_____ _____ _____