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Dietary Folate, Other B-Vitamins and Incident Alzheimer's Disease: The Cache County Memory, Health, and Aging Study

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DIETARY FOLATE, OTHER B-VITAMINS AND INCIDENT ALZHEIMER'S DISEASE: THE CACHE COUNTY MEMORY, HEALTH AND AGING STUDY

by

Chailyn Nelson

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ABSTRACT

Dietary Folate, Other B-vitamins and Incident Alzheimer's Disease:

the Cache County Memory, Health, and Aging Study

by

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Utah State University, 2008

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This study involves data from the Cache County Study, which began in 1994 with joined efforts by Duke University, Utah State University, and Johns Hopkins University. It consisted of 5,092 participants from Cache County, Utah, located in the northern part of the state. Characteristics of the population include high participation rates (~ 90%), a majority of participants are members of The Church of Jesus Christ of Latter-day Saints, longer life expectancy than the general US population, a greater than 80% rate of at least a high school education, and low rates of migration.

Subjects' cognitive status was screened using the Modified Mini-Mental State Examination or rated by knowledgeable informants using an Informant Questionnaire for Cognitive Decline. Low scoring subjects were examined using the Dementia Questionnaire, an inventory of cognitive symptoms, functional impairments, and medical conditions relevant to dementia. The clinical data were reviewed by a geropsychiatrist and neuropsychologist. Those suspected of dementia underwent further testing and final

dementia diagnoses were decided by a consensus conference of experts. Clinical assessment at the baseline interview identified 368 individuals out of the original 5,092 subjects as having dementia. These individuals were removed from the present analysis. Prevalent cases of dementia were excluded in our analyses of risk associated with incident AD.

Dietary data were collected using a food frequency questionnaire at baseline in 1995. A list of 142 foods was provided and participants noted frequency they consume the food or food group. To calculate intake of a specific nutrient, the nutrient content of each food is multiplied by the frequency of consumption for each food. This number is summed over all food items.

Cox Proportional hazards modeling was used to assess risk of incident AD in relationship to folate and B-vitamin intake over eleven subsequent years of data collection. Cox modeling assists in analysis of censored cases (drop-outs and deaths). No relationship was found between folate from food, supplement, or combined sources with dementia or with AD. Similar results were observed for B-12 and B-6.

(129 pages)

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Chailyn Nelson

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CHAPTER 1

BACKGROUND AND INTRODUCTION

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease with devastating and debilitating consequences (1). Diet is among several modifiable factors that likely play a role in an individual's risk of developing AD (2, 3). B-vitamins such as folate, vitamin B-12, and vitamin B-6 are particularly interesting due to their role in one carbon transfers or methylation reactions including homocysteine metabolism (4). Deficiencies of B-vitamins are known to cause elevated levels of homocysteine and hyperhomocysteinaemia has been observed at high rates among individuals with dementia (4). Elderly men and women have increased risk for B-vitamin deficiency due to malabsorption of these nutrients caused by gastritis, interaction with medications, and conditions like pernicious anemia (5, 6). Since the mandate for folate fortification of the US food supply in 1998, folate's potential to mask a B-12 deficiency has become more of a concern for this population as prolonged vitamin B-12 deficiency can result in irreversible neuronal damage (6, 7). The results of previous studies examining B-vitamins and cognitive function are inconclusive. The objective of this thesis is to examine associations between B-vitamins and incidence of Alzheimer's disease in a cohort of aging men and women of the Cache County Memory Study.

BACKGROUND

Alzheimer's disease defined

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that results in shrinkage of the frontal and temporal lobes of the brain effecting emotions, learning and memory functions (8). AD is the most common cause of dementia and accounts for approximately 70% of all dementia cases (9). Some critical components used to diagnosed AD, though the criteria vary, are a decline in cognition including memory loss, impairment in performing activities of daily living and a progressive pattern of decline and impairment (10).

Cost of Alzheimer's disease

Alzheimer's disease significantly reduces quality of life in affected individuals and their caretakers. In 2004, it was reported that Alzheimer's disease (AD) affected nearly 2% of people living in industrialized countries (8). With the aging of the Western populations, it has been reported that the incidence of the disease is expected to more than double by 2050 (8, 11). It is suspected that this is mostly due to the aging of the US population. As the baby boomers (born in 1946-1964) age there will be more people alive at the high-risk ages (11)

AD increased the cost of health care by an estimated $80 to $100 billion dollars in 2003 (3). It ranks third behind heart disease and cancer for most costly disease in the US (3). In 1991, Medicaid spent $5.7 billion dollars in care for AD patients and in 1996, the estimated cost for caring for a patient with AD was about $18,000-$36,000 per year depending upon severity (12, 13). Though researchers have learned much about the

disease in the last few years, there are still many uncertainties surrounding the risk factors, causes, and effective treatment of AD (3).

Delaying or slowing the progression of AD would lead to substantial savings in healthcare and extended periods of high quality life. In 1998, a one month delay in need for institutionalized care for dementia and AD patients would yield savings of $1,863 in formal services per individual per year and collectively $1.12 billion annually (13). It is obvious that AD is one of the most costly and fastest growing public health problems of today, and that delaying onset could have significant financial benefits. More importantly delaying onset or slowing progression may help elders live independently for a longer period of time and decrease caregiver burdens.

Etiology and pathology

The pathology behind neuron cell death and cognitive decline is not completely understood. There are several theories and possible mechanisms that likely contribute to normal synapse failure and neuron death. "Plaques" made of amyloid β-peptide accumulate between nerve cells and "tangles" made of fibrillar substances made of the tau protein form within nerve cells are thought to increase oxidative stress that damages DNA, proteins, and lipids of neurons in the brain (8). When phosphorylated fibrillary proteins accumulate; these are known as the "tangles." Moderate or mild build-up of tangles is associated with normal aging processes, but increased numbers may be a cause of neuron death associated with AD (10).

Whereas tangles are intracellular lesions, senile plaques form extracellularly. Amyloid material derived from a precursor protein called amyloid-beta precursor protein

(APP), can be deposited on neurons causing dystrophic neurites (1). The normal form of APP has many functions; one role is thought to be in neural growth and repair (14). Dystrophic neurites exhibit "distorted" neuron processes or abnormal synapse processes (10). The proportion of individuals with plaques increases with age and by the 80th decade of life, 75% of the population has at least some degree of plaque concentration, although a high degree of variation exists among individuals, even among the oldest old. Plaques begin as non-aggregated and harmless deposits but in some individuals a transformation occurs and innocuous plaques become dystrophic plaques associated with AD. The exact cause of this transformation is unknown, some theorize the enzyme butyrycholinesterase is involved (10). Though older individuals are more likely to have plaques, the number of plaques is relatively low in elderly with normal cognition (10).

The amyloid cascade begins with antecedent events such as genetics, environment and lifestyle contributing to amyloid production. Enzymes called secretases including the alpha, beta, and gamma type involved in splicing protein segments that form APP, play a large role in whether a deposition of amyloid occurs in excess or not. This brings up interesting questions in the field of genetics; those with genotypes for certain types of secretases may have more or less predisposition for AD (8). The dysfunction of neurons is not the only theorized cause of cognitive decline. Amyloid buildup is thought to cause inflammation, oxidative stress and to impair neurogenesis (8).

The order in which these events happen (amyloid deposition, inflammation, oxidative damage, etc.) and how they contribute to the etiology of the disease is often debated. Some believe that plaques cause inflammation and oxidative damage others believe the oxidative damage occurs prior to plaque formation. The latter theory is

derived from the hypothesis that oxidative stress, through a series of enzymatic steps, may cause alterations in the processing of APP or tau leading to plaques and tangles. In an article discussing mitochondria and neurodegenerative diseases, it is said that mutations in mitochondria DNA contribute to diseases like AD. As people age they accumulate more mutations which may lead to increased ROS production (15).

Contributing factors

Alzheimer's disease has been found to be associated with a variety of environmental and inherited factors. Non-modifiable factors include age, gender and apoE genotype. Modifiable factors include diet, physical activity, education, etc. Though AD is strongly associated with age and genetic risk, it is well accepted that many additional factors such as diet and exercise have a large impact on risk for and progression of the disease (3).

Prevention and treatment

Pharmaceuticals targeting different areas of the neurodegenerative cascade have been developed and tested. For example, blockers of neurotoxic substances and drugs that chelate copper and iron have been tested. Non-steroidal anti-inflammatory agents and substances that may cue the immune system to remove harmful matter from the brain are also being explored (8). Common pharmaceuticals like cholinesterase have been found to provide modest effects, but are often accompanied with severe gastrointestinal side effects (16). Due to the variability in effectiveness and relatively mild improvements made by pharmaceutical agents, more preventive measures need to be defined, including diet modifications which might reduce earlier and faster cognitive decline.

The study of diet and AD has boasted a lot of attention the last few years; interestingly, this coincides with the projected increase in prevalence of AD. The New Mexico Aging Process Study is a longitudinal study which began in 1979. A paper reporting on nutrition status and cognition was done on the cohort using data from a 6-year reassessment. The cohort included 137 elderly who were well educated, healthy, financially secure, and generally health conscious. Three day diet records, biochemical, anthropometric and clinical measurements were taken. The results included a modest relationship between nutrition status and cognitive decline. Nutrition status was based on dietary intake and correlated biochemical markers of vitamin c, thiamine, riboflavin, niacin, folate, vitamin B-6, vitamin B-12, protein, and iron. The positive associations were noted for protein, folate, niacin, ascorbate, vitamins B-6 and B-12, thiamine, and riboflavin (17).

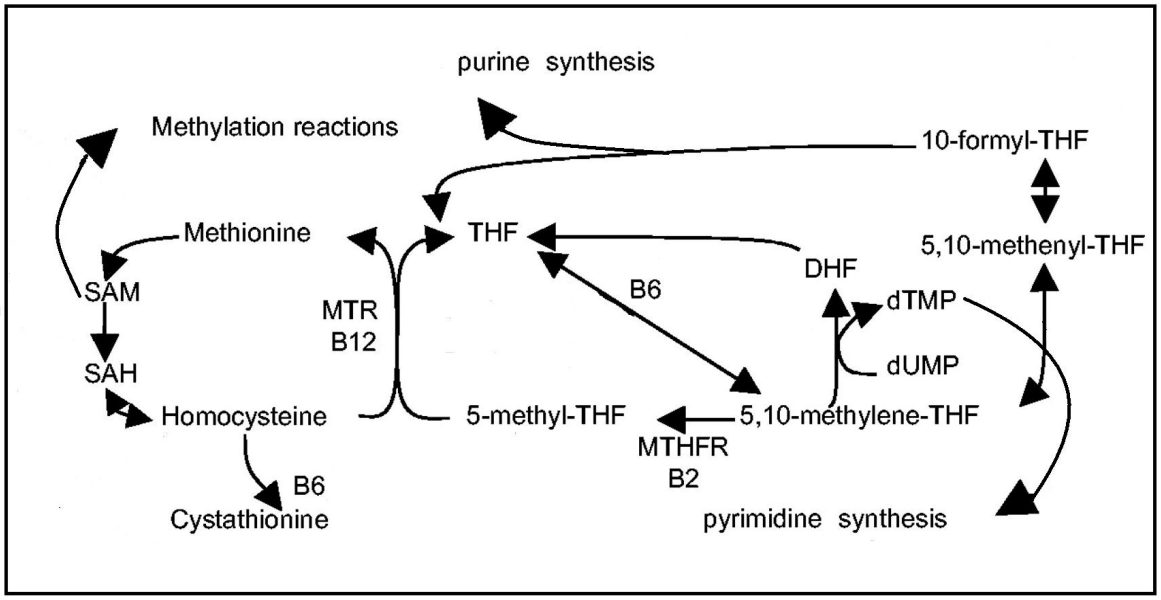

FIGURE 1.1 One-Carbon Metabolism (Adapted from Lim, 2005) (18)

One-carbon metabolism and B-vitamins

The figure above (**Figure 1.1)** shows the interrelationsthips between B-vitamins and homocysteine in methylation reactions including the formation of methionine for S-adenosyl-methionine (the methyl donor for essential reactions in the brain) (9). It is clear that for these reactions to function properly folate must be present. In one-carbon metabolism which is the transfer of methyl groups from donors to acceptors, folate as a part of 5-methyl-tetrahydrofolate assists in the methylation of homocysteine to methionine along with the enzyme methionine synthase and vitamin B-12. This step of methionine production from homocysteine is very important as it provides a substrate for SAM (9).

B-12 is a required cofactor for the remethylation of homocysteine to methionine (9). A deficiency of SAM may also occur with deficiency of B-12 even in the presence of adequate folate. The conversion of 5,10 methylene-THF to 5-methyl-THF, the methyl donor for homocysteine, requires the enzyme methyltetrahydrofolate reductase. This reaction is irreversible; therefore B-12 deficiency leading to 5-methyl-THF excess is known as the methyl-folate trap. This trapping of folate as 5-methyl-THF also results in a lack of folate precursors needed for DNA synthesis and red blood cell maturation (7).

Vitamin B-6's role in one-carbon metabolism is as a cofactor for that metabolize homocysteine through an alternate pathway and as a cofactor for serine transhydroxy-methylase between tetrahydrofolate (THF) and 5, 10 methylene-THF. Homocysteine may be converted into several different products including: 1) methionine, 2) cystathionine, and 3) homocysteine sulfinic acid. Vitamin B-6 is involved in the conversion of homocysteine to both cystathionine and homocysteine sulfinic acid (9).

Mechanistically deficiency of folate, vitamin B-12 and vitamin B-6 independent of each other may result in excess homocysteine, deficiency in SAM, and a lack of folate precursors involved in DNA synthesis and red blood cell maturation. The status of one may affect the importance of other B-vitamins. For example, B-6 may become very important in the presence of other B-vitamin deficiencies. In a rat model, B-6 deficient rats were seen to have adverse increases in homocysteine compared to the folate deficient rats (19). In addition, adding methionine resulted in increased SAM in folate deficient rats and signaled further homocysteine catabolism, indicating that B-6 may be critical for homocysteine metabolism in the presence of folate deficiency. In the B-6 deficient rats, an increase in SAM prevented homocysteine remethylation therefore increases in plasma homocysteine levels (19).

In addition to folate, vitamin B-12, vitamin B-6, there are other nutrients including riboflavin, betaine, and zinc that are important for one-carbon metabolism. A few publications note riboflavin's involvement related to its role in genetic predisposition to low folate status and/or high total homocysteine (tHcy) levels (20). Riboflavin is a cofactor to methyltetrahydrofolate reductase (Figure 1.1), an enzyme required for conversion of 5,10 methylene-THF to 5-methyl-THF. Little research examining interactions between riboflavin, folate, B-12, B-6 and cognition is available. Betaine, a metabolite of choline, has also been mentioned in this area due to its involvement in an alternate methlyation pathway of homocysteine; this occurs mainly in the liver and little research has been done involving betaine (4). Niacin and thiamine deficiencies are now rare in the US, but also have neurologic effects during deficiency. Pellagra, caused by niacin deficiency is known to cause dementia, diarrhea, and dermatitis. Wernicke-

Korsakoff syndrome, is the result of thiamine deficiency often seen in alcoholics, and is associated with memory loss and hallucinations. Thiamine deficiency results in macrocytic anemia but no neurologic syndromes have been identified (21). Niacin and thiamine have received less attention in major cognitive studies.

One-carbon metabolism and AD

Dysfunction of one-carbon metabolism may contribute to cerebral atrophy and neuronal cell death (22). Disturbed one-carbon metabolism could be linked in several ways to AD. Although details regarding these proposed mechanisms are unclear, prolonged folate and B-12 deficiency may lead to hyperhomocysteinaemia. Elevated levels are generally considered to increase oxidative stress leading to damaged neurons (10, 23). In several studies elevated homocysteine has been found to be associated with increased risk for AD or to faster cognitive decline (4).

Another potential mechanism associating one-carbon metabolism and AD exists via a deficiency of SAM. SAM deficiency may also result from folate or B-12 deficiency or both, preventing homocysteine from being methylated to methionine, the precursor for SAM (Figure 1.1). Inadequate SAM alters normal methylation of nucleoproteins, proteins, membrane phospholipids, and neurotransmitters essential for normal brain function (4, 9, 24, 25). In addition to this, SAM is converted to S-adenosylhomocysteine (SAH) which is hydrolyzed to homocysteine. When homocysteine metabolism is halted SAH surplus is favored, and SAH may inhibit several enzymes of SAM metabolism (26). Like homocysteine and B-vitamin levels in AD patients, SAM has also been

observed at low serum levels in patients with AD and postmortem in brain tissue of AD patients (24).

In addition, through a series of reactions beginning with metabolites in one-carbon metabolism glutathione is produced. Glutathione is a major contributor to reduction of oxidative stress in the brain, and reduced glutathione stores have been observed in AD patients. Some of the reactions leading to production of glutathione require vitamin B-6 and SAM (23). Finally, specific folate metabolites are required for pyrimidine and purine synthesis (4). Deficiencies of these metabolites can be caused by methyl-folate trap (previously discussed) and may cause mutations or breaks in DNA. Damaged DNA has been implicated in cancers and neurologic defects (27).

Folate's roles

Folate acts as a cofactor for several enzymes that aid in prevention of anemia, cardiovascular disease, thromboembolitic processes, neural tube defects and other congenital defects, adverse pregnancy outcomes, neuropsychiatric disorders, and cancer (28). Folic acid is the fully oxidized monoglutamyl form of the vitamin and is used commercially in supplements and in fortified foods (28). Though folate has received the most attention of the B-vitamins in relation to cognition, other B-vitamins such as vitamins B-12 and B-6 and their role in cognitive health should not be discounted. If folate deficiency was the only B-vitamin deficiency involved, a decline or slowing in the rates of AD with folate fortification would have been expected. No evidence exists that a decrease in AD rates has occurred, although this may be related to poor surveillance of AD rates.

Vitamin B-12, neuropathy and macrocytic anemia

Vitamin B-12 deficiency is often accompanied by hematologic, neurologic (paresthesias, peripheral neuropathy, demyelination), psychiatric (irritability, mild memory impairment, dementia, depression, psychosis), and possible cardiovascular symptoms (29). Deficiency of B-12 has been found to be related to neurologic damage due to altered methylation reactions (30). Vitamin B-12 is also required for the conversion of methylmalonic acid to succinyl-CoA. Deficiency in B-12 leads to elevated methylmalonic acid (29). Some studies have proposed methylmalonic aciduria may damage mitochondria involved in mitochondrial oxidative phosphorylation and this appears to effect brain tissue. It may also inhibit kinase reactions in the brain, by inhibiting mitochondria creatine kinase in the cerebral cortex (31). A deficiency of either folate or vitamin B-12 also leads to macrocytic anemia but isolated B-12 deficiency results in neurological symptoms and can occur in the absence of anemia, the primary indicator of deficiency (5).

Prevalence of B-vitamin deficiencies

A high prevalence of folate and B-12 deficiency is observed among older people in the US. Vitamin B-12 deficiency rates were found to increase with age in three studies of elderly living in the United Kingdom and on average 1/20 age 65-74 were deficient and 1/10 age 75+ were deficient (5). The review of studies done on UK elderly also found folate deficiency to increase with age but only 10% of people with low B-12 also had low folate levels. The blood samples were drawn during the later half of the 1990's from all three studies (5). Kim (28) states vitamin B-12 deficiency may be the larger

problem and may affect up to 10-15% of the population over 60 years of age. Deficiency of B-12 is commonly associated with hypochlorhydria, pernicious anemia, and atrophic gastritis (5).

Vitamin B-12 is absorbed in the small intestine. Hydrochloric acid (HCl) in the stomach releases B-12 from protein. In the duodenum, B-12 is then bound to intrinsic factor (IF), a factor released from parietal cells of the stomach. The B-12-IF complex is absorbed in the ileum of the small intestine. Inadequate IF for B-12 binding and absorption is often a result of the autoimmune response called pernicious anemia, and may ultimately lead to B-12 malabsorption (29). Conditions which decrease HCl levels, such as gastritis and hypochorhydria associated with medications, prevent B-12 from being released from food proteins and ultimately prevent absorption of B-12 (5).

Assessment of B-vitamin nutriture

Literature currently indicates that serum B-12 is a poor marker for detecting B-12 adequacy (21, 32). Biomarkers like methylmalonic acid (MMA) and holotranscolbalamin have been recommended as more sensitive indicators. MMA levels above 376 nmol/L appear to be indicative of B-12 deficiency as well as serum holotranscobalamin greater than 45 pmol/L. Holotranscobalamin is stated to be a useful measure to detect earlier B-12 imbalances (33).

When assessing B-12 status using holotranscobalamin or MMA, creatinine must be taken into account. Creatinine is a product created from the breakdown of creatine a component of muscle and is often an indicator of kidney function (34). Hvas and Nexo (34) reported that increases in serum creatinine by 100 µmol/L doubled MMA,

holotranscobalamin, and other B-12 metabolites. Therefore, assessment of B-12 should include corrections for creatinine level, as creatinine is often elevated for reasons not related to vitamin deficiency.

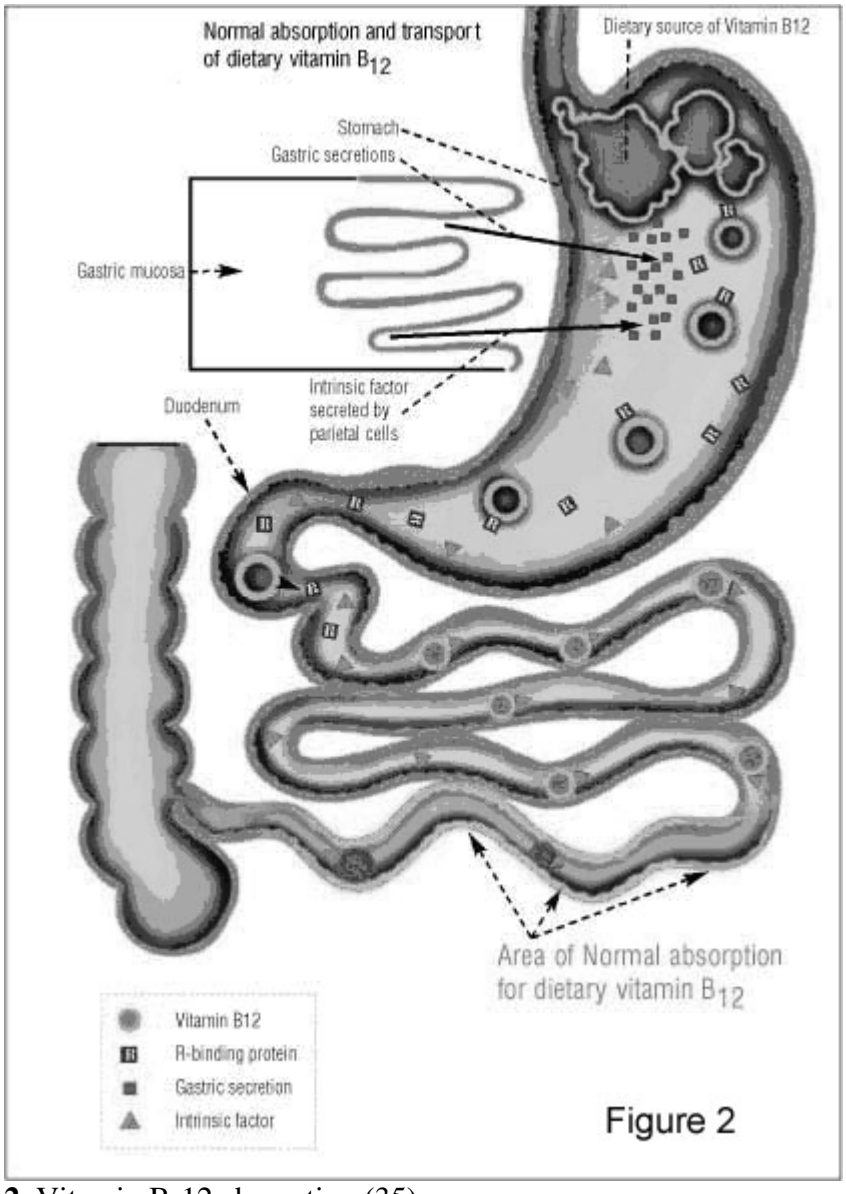

FIGURE 1.2 Vitamin B-12 absorption (35)

In 2000, the prevalence of gastritis was estimated to be between 20%-50% of elderly based on the diagnosis criteria used. The Framingham study found 24% of individuals 60-69 years old and 37% of individuals over the age of 80 to had gastritis. It is important to note that only in the most severe cases of gastritis does intrinsic factor secretion become limited to the point of vitamin B-12 malabsorption. Gastritis may lead to bacterial overgrowth which can also affect absorption of B-12 due to the bacteria's uptake of the vitamin. The condition can also affect folate absorption by decreasing hydrochloric acid in the stomach therefore, increasing the pH in the stomach and small intestine. The slight increase in pH can make a noteworthy decrease in releasing B-12 from protein and decreases folate absorption (26). Lower levels of B-vitamins and higher homocysteine levels are also common in the elderly (36).

Folate fortification

In 1998, in response to overwhelming evidence that levels of folate was associated with increased risk for neural tube defects, all flour and uncooked cereal-grain products in the US were required to be fortified with folic acid at a level of 140 micrograms/100 grams (28, 37). In response to folate fortification, it is estimated that neural tube defects prevalence and incidence has decreased 15-50% in the US (28). In the Framingham cohorts it was reported that subjects who did not take supplements with B-vitamin had 38% higher RBC folate post folate fortification (38). Prior to fortification, 4.9% were deficient in folate and post-fortification only 1.9% folate deficient. The authors noted that this increase in serum folate may be masking B-12 deficiencies and causing irreversible neurologic damage (38).

In the face of concerns that high levels of folate may mask B-12 deficiency in elderly, the 140 µg of folate fortification level was chosen with the intent that only a few people would exceed 1 mg per day. The upper limit of 1 mg per day was somewhat arbitrarily selected by the Institute of Medicine as an improbable level to produce masking. Kim (28) stated that this is controversial due to the fact that others argue intake less than 1 mg may result in the masking of a B-12 deficiency. Kim also states that other countries have failed to follow the US in fortifying with folate due to this debate despite its obvious benefits among pregnancy and birth defects (28).

It should be noted that 50-95% of folate content in food is estimated to be lost in storage, preparation, or manufacturing processes. And some have questioned whether the average person in the US is receiving adequate folate even post-fortification (39). One study found that though dietary intake of folate was low that serum levels were not below normal indicating that the subjects were not yet in negative nutrient balance. It is difficult to assess B-vitamins intake from food because there are many problems with getting accurate measurements of folate intake. There are over 150 different forms of folate and determining bioavailable portions in different foods has variable accuracy (39). Bioavailability of food folate has been reported to vary anywhere from 10-98% (40). Exact differences in absorption between folate fortified foods and folate naturally occurring in foods is also unknown. It appears synthetic forms of folic acid whether fortified to food or in supplement form are more bioavailable than folate from food (41).

Folate and B-12 masking

Because interactions between B-12 and folate were considered potentially problematic, the level of fortification was chosen to be 140 micrograms of additional folic acid per day. Given typical consumption patterns, this level of fortification should result in very few instances of intake greater than 1 mg, the level set by the Institute of Medicine as the upper limit, and at which masking of B-12 deficiency may be problematic. This has not been proven and there is inconsistency in actual evidence identifying the level where masking would likely occur (28).

Morris et al. (7) found that 4% of their study's participants, who included elderly men and women from the US National Health and Nutrition Examination Survey, had both a low vitamin B-12 and high folate status. This status was related to increased risk for anemia and greater cognitive impairment. Interestingly, when B-12 status was normal, high serum folate was associated with protection against cognitive impairment rather than possible harm (7). If 4% of the elderly in the US are similar to this subsample, then ~1.8 million may be at risk for decreased cognition related to folate and B-12 imbalances (42).

Smith (42) presents multiple questions on issues surrounding this topic, such as the questions*:* "should the issue of fortifying food with vitamin B-12 be reopened in those countries that have already fortified certain foods with folic acid?" and "should countries considering folic acid fortification defer a decision until more is known about the interaction between folate and vitamin B-12 status?" (42). Though the main focus of our research is to shed light on B-vitamins and AD, results from this project may provide insight about this issue.

Though some studies have identified association between B-vitamins and cognition, these findings are far from consistent, and several, including some who have examined these associations in large prospective studies, have observed no association between dietary folate and B-12 and cognition or AD incidence (30, 43-49).

INTRODUCTION TO THE CACHE COUNTY MEMORY, HEALTH, AND AGING STUDY

The Cache County Study began in 1994 with joined efforts by Duke University, Utah State University and Johns Hopkins University. It included 5,092 participants from Cache County, Utah, located in the northern part of the state. As a longitudinal cohort study with four waves of data collected presently, the Cache study has many strengths. One strength of the Cache study is its high rates of participation with 89.9% of those eligible participating at baseline to 79% during last wave. Participants of the Cache County Study were men and women 65 years of age or older in 1995. Most (91%) of the participants are members of The Church of Jesus Christ of Latter-day Saints (LDS). This religion urges its members to practice healthy behaviors such as excluding the use of tobacco and alcohol. Not surprising, the population has a longer life expectancy than the general US population. The 1990 U.S. Census reported the median life expectancy in Cache County at age 65 was highest in the nation, almost ten years greater than the national average (50, 51). This population is also well educated with more than 80% having completed at least a high school education. The Cache population also has lower rates of migration and is known for large close families, factors helpful for participant retention throughout the study.

The original purpose of the study was to examine the prevalence and incidence of dementia and by cognitive screening and careful clinical assessment of the participants over a period of time. Requirements for participation in the study included a history void of a central nervous system disorders or current psychiatric disorder and completion of a dementia screening. At baseline, 4,298 subjects were dementia-free. Approval from institutional review boards at USU, Duke, and Johns Hopkins were obtained, and each participant signed an informed consent form before participating.

Each subject's cognitive status was screened at the baseline and subsequent follow-ups using the Modified Mini-Mental State Examination (3MS) or rated by knowledgeable informants using an Informant Questionnaire for Cognitive Decline (IQCODE). The genotype at the locus for Apolipoprotein E (APOE) gene was determined from DNA from a buccal scraping collected from subjects. Subjects scoring less than 87 points on the 3MS or higher than 3.27 on IQCODE and all subjects older than 90 years were further examined in clinical follow-up using the Dementia Questionnaire (DQ) administered by trained nurses. The DQ is an inventory of cognitive symptoms, functional impairments, and medical conditions relevant to dementia. The suspected cognitive impaired group was supplemented with a sample of individuals randomly selected from those deemed non-demented at baseline and both groups were asked to complete a comprehensive clinical assessment that included examination, blood-pressure, neurologic examination, psychometric testing, and review of cognitive symptoms and medical histories.

The clinical data was reviewed by a geropsychiatrist and neuropsychologist who then assigned diagnoses of dementia, other cognitive disorders, or normal cognitive

status. Those suspected of dementia underwent further testing and final dementia diagnoses were decided by a consensus conference of experts including a neuropsychologist, a board-certified geriatric psychiatrist and the examining nurse and technician.

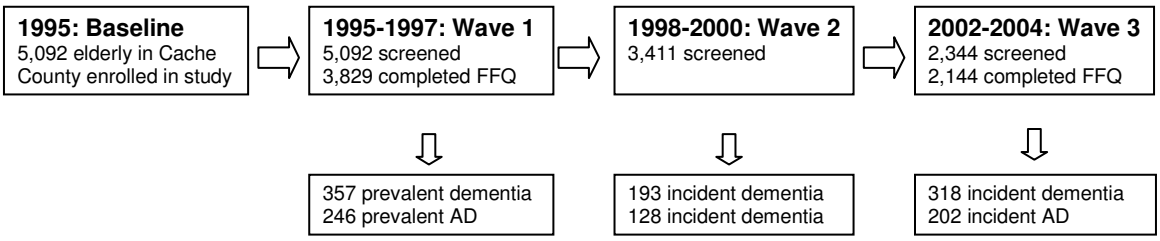

FIGURE 1.3 Cache County study design

Clinical assessment at the baseline interview identified 368 individuals out of the original 5,092 subjects as having dementia. Prevalent cases of dementia will be excluded in our analyses of risk associated with incident AD.

Usual dietary intake was assessed using a food frequency questionnaire (FFQ) at baseline (in 1995) and during a follow-up assessment (wave 3) in 2002-2004 (see appendix A and B). The FFQ used was modeled after the Harvard Nurses' Health Study FFQ, and its format has been validated for replicated uses. FFQ's are useful for large population-based studies because they can be administered by an interviewer (wave 3) or done by the participant by themselves (wave 1). They are relatively inexpensive and are a quick method for assessing usual intake, using only one inquiry. A list of 142 foods or groups of foods were provided and participants noted the frequency with which they consumed the food or food group by the following categories: less than 1 per month, 1-3

times per month, 2-4 times per month, 1 time per week, 2-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, and 6 or more times per day.

To calculate intake of a specific nutrient, the nutrient content of each food per 100 grams was multiplied by the frequency of daily consumption of each food. This number was then summed over all food items. A database containing nutrient content of foods reported by participants in the CCMS with nutrients per 100 grams was created using a standard nutrient database, the Food Processor created by ESHA Research and data from food manufacturers (52). The Food Processor version 7.02 (1997) consists of over 30,000 foods (there are >32,000 foods in more current versions) and is updated frequently. It includes specific brands and types of foods such as "Wendy's chili" or "Kellogg's Corn Flakes," although many of these foods are not available through the USDA. Approximately ¼ of the foods come from the USDA database, the rest come from manufacturers and restaurants. The Food Processor also allows new recipes to be entered and version 8.2, which food composition data should correlate with wave one data has 45 nutritional components available for analysis (more recent versions have closer to 164) (52). We created a food table of the food choices seen on the FFQ to be used to calculate daily intakes.

The software FoodCalc, was used for analysis and quantification of the FFQ data. The FoodCalc program is public-domain software from the Danish Cancer Registry written by Jesper Lauritsen, and is an effective tool for evaluating large amount of FFQ data (53). Once a table of foods representative of the foods in the FFQ was compiled and computed per 100 grams, Foodcalc was used to total nutrients by individual's intake

per day, by specific foods or food groups, and overall totals by all individuals for all foods consumed (53).

RESEARCH QUESTIONS

The analyses presented in this thesis will attempt to address the following research questions:

1. Are dietary intakes of B-vitamins including folate, vitamin B-12, and vitamin B-6, from both food and supplemental sources associated with incidence of dementia and AD?
2. Do interactions between intakes of these nutrients mediate the association of individual B-vitamins and incidence of AD?
3. Are these associations modified by potential confounders such as gender, age, education level, other dietary and health behaviors, and apoe-4 genotype?

OBJECTIVES

The objectives of this study are as follows:

1. Create a nutrient database of all foods reportedly consumed in wave 1 and wave 3 of the Cache County Memory Study with measurement of nutrients per 100 gram servings. Use the Foodcalc program to compute the amount of nutrients consumed per day for each individual.
2. Examine associations between food folate, supplemental folate, and total folate with incident dementia and incident AD.
3. Investigate effects of varying intake levels vitamin B-12 and folate with AD controlling for B-6. Examine associations accounting for confounding factors

such as age, sex, education, APOE genotype, weight status, history of physical activity, history of comorbidities, and other dietary factors.

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CHAPTER 2

LITERATURE REVIEW OF THE RELATIONSHIP BETWEEN FOLATE, OTHER B-VITAMINS AND ALZHEIMER'S DISEASE

ABSTRACT

The influence of dietary intakes and nutrition status on cognitive health in the elderly has been the focus of much research. Folate and other B-vitamins have specifically been considered due to their known roles in one-carbon metabolism. Mechanistically it is possible these nutrients may also affect incidence of Alzheimer's disease (AD) by contributing to decreased production of S-adenosyl methionine (the primary methyl donor for the brain) formation, elevated homocysteine (a possible neurotoxin) and through abnormal DNA production (which requires certain forms of folate). Elderly populations have higher rates of folate and B-vitamin deficiencies than other age groups and may contribute to higher rates of elevated serum homocysteine observed among elderly. Hyperhomocysteinemia has been found to be associated with cognitive impairment and AD. Whether elevated levels of homocysteine are merely a marker of poor diet or insufficient folate and B-vitamins or a contributing factor for development of AD is unclear. Studies of associations between dietary intake and serum levels of B-vitamins, homocysteine, cognitive decline, and incidence of AD have reported inconsistent findings. Dietary folate, B-12, and B-6 have been studied as possible independent risk factors for cognitive health, but it may be possible that the effects these micronutrients have on cognitive health is mediated by interactions between these nutrients.

RELATIONSHIP BETWEEN FOLATE, B-VITAMINS AND ALZHEIMER'S DISEASE

Introduction

Many of the roles of folate, vitamin B-12 and vitamin B-6 in one-carbon metabolism have long been well established. The relationship between these micronutrients and homocysteine levels has been observed in many studies, and elevated homocysteine has been associated with several diseases including heart disease and Alzheimer's disease (AD) (1, 2). However, it is unclear whether elevated homocysteine is a marker or risk factor of disease. Whether folate, B-12 and B-6 status are independent risk factors of cognitive decline via metabolism of homocysteine or other metabolites and ultimately Alzheimer's disease is unknown. It is possible that the existence of the disease may result in poorer diet, which would offer an alternative explanation to the observed associations of lower serum levels of folate, B-12 and B-6 in individuals with AD.

It is particularly interesting to examine relationships between AD and B-vitamins among elderly populations for two obvious reasons. First, AD is a disease of aging and occurs at higher rates with increasing age. Secondly, elderly populations have increased rates of deficiencies of B-vitamins. Vitamin B-12 deficiency is of particular concern among elderly populations due to high rates of food bound malabsorption of B-12. B-12 must be released from proteins by acids in the gastrointestinal tract, decreases in acidity caused by gastritis, medication, and other conditions in this population prevent absorption of vitamin B-12. Gastritis is more commonly seen in elderly due to increased bacterial overgrowth (3) and leads to increased use of gastric-acid blocking medications (4) which decrease the release of B-12 from food. This is not an issue with dietary supplement

intakes of B-12. Elderly also have higher rates of pernicious anemia, an autoimmune reaction where intrinsic factor (IF) production is decreased. After B-12 is released from proteins, IF binds B-12 making it available to be transported across the gut membrane (4). Pernicious anemia decreases absorption of both food and supplemental forms of B-12.

The association between folate, vitamin B-12, vitamin B-6 and cognitive health is not widely agreed upon. Researchers have observed positive associations, no association, and occasionally negative associations. Some studies suggest that higher intake of folate is protective against cognitive decline (5, 6); others report possible harmful effects of higher folate intake (6, 7). Most researchers in the field agree that more prospective studies with adequate power are required to bring further light to the discrepancies observed thus far.

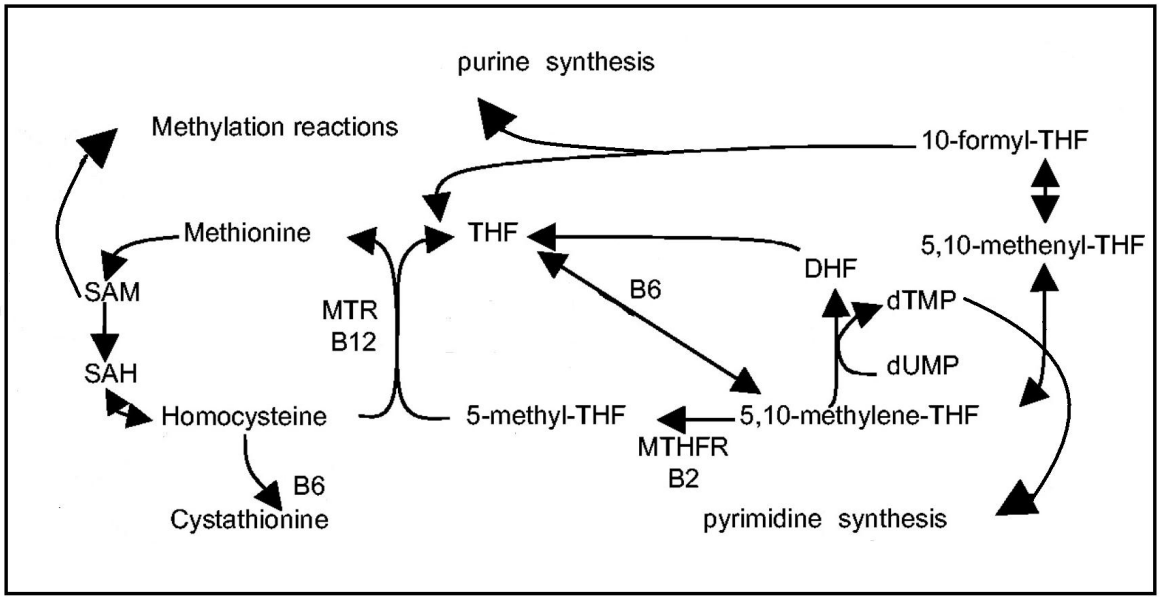

FIGURE 2.1 One-carbon metabolism (Adapted from Lim, 2005) (8)

Proposed Mechanisms

The relationships between B-vitamins and one-carbon metabolism are evident in many reactions of one-carbon metabolism (see **Figure 2.1**). Key steps where B-vitamins are involved include folate and B-12 role in the methylation of homocysteine to methionine; which in turn is converted into S-adenosyl-methionine (SAM), a methyl donor for many reactions. B-6 is required for alternate pathways of homocysteine metabolism and folate cycling (9). The conversion of 5,10-methylene-THF to 5-methyl-THF, the methyl donor for homocysteine, is catalyzed by the enzyme methyltetrahydrofolate reductase (MTHFR). This reaction is irreversible and the conversion of 5-methyl-THF to THF is dependent on vitamin B-12. Therefore, B-12 deficiency traps folate as 5-methyl-THF and is appropriately known as the methyl-folate trap. This condition of B-12 deficiency and adequate folate also results in a lack of coenzyme forms of folate needed for DNA synthesis and red blood cell maturation (10, 11).

Vitamin B-6 may become more or less important to homocysteine metabolism depending of status of other B-vitamins. B-6 acts as a cofactor to an alternative pathway of homocysteine metabolism; homocysteine is converted to cystathionine rather than methionine in this reaction. In one experiment, folate deficient rats were loaded with methionine which increased SAM and in turn signaled homocysteine catabolism despite inadequate folate. In rats who were also B-6 deficient, the upswing of SAM was not accompanied by homocysteine remethylation; indicating B-6 may be crucial for maintaining homocysteine equilibrium in folate deficient states (12).

Deficiencies of B-vitamins leading to disturbed one-carbon metabolism are theorized to enhance AD progression or cognitive decline in multiple ways. The most prominent association between one-carbon metabolism and AD is probably the theory that elevated homocysteine induces oxidative stress which may lead to neurotoxicity (13, 14). Hyperhomocysteinaemia, may result from deficiencies of B-vitamins singularly or in combination and has been observed in numerous studies concerned with cognition (6, 9).

The prevention of homocysteine remethylation to methionine also leads to decreased formation of SAM. SAM deficiency in turn may disrupt a number of key methylation reactions required for normal brain functioning affecting proteins, membrane phospholipids, neurotransmitters, and DNA structure (6, 9, 15, 16). The functioning of SAM has been observed to be inhibited by surplus of S-adenosylhomocysteine (SAH) which is also caused by halted homocysteine metabolism (1). SAH has also been observed at abnormal levels in individuals with AD similar to homocysteine and B-vitamins like folate, B-12, and B-6 (15).

Glutathione is a key antioxidant with the brain and is formed from a series of reactions that originate with homocysteine. Several of the reactions leading to production of glutathione require vitamin B-6 and SAM. This is another potential source of increased neural damage linked to one-carbon metabolism (13). Finally, as was briefly mentioned earlier B-vitamin deficiencies may lead to what is known as the methyl-folate trap. This may limit availability of folate metabolites needed for DNA formation and has been implication in breaks in DNA was may contribute to neurologic defects (6, 17).

Diet and serum biomarkers of B-vitamin status

Studies involving dietary intakes of B-vitamins and related serum outcomes have established strong relationships. In 1996, Tucker et al. (18) published their examination of the relationship between the dietary intakes and plasma levels of folate and homocysteine in the Framingham cohort. The top contributors to dietary folate were breakfast cereal, fruit and vegetable intake, and other fortified foods. They found clear dose relationships between increasing quintiles of breakfast cereal, fruit and vegetable intake, plasma folate and lower levels of homocysteine (18).

A study done by Tucker et al. published in 2004 (19) examined the relationship of B-vitamin intake and homocysteine levels in a trial involving varying levels of fortified ready-to-eat-cereal. The 215 subjects were age 55-85 and had no history of hypertension, anemia, asthma, cancer or cardiovascular disease or digestive disease and no regularly consumption of multi-vitamin mineral supplements, B-vitamin supplements, or highly fortified breakfast cereal. The subjects were randomly divided into intervention and placebo groups; the intervention group received a fortified cereal (440 µg folate, 1.8 mg vitamin B-6, and 4.8 microgram B-12) for 12 weeks and the placebo a cereal similar in calories, fiber, carbohydrate, and other B-vitamins for the same time period. The results showed a significant difference in homocysteine levels between the two groups. Homocysteine concentrations were significantly lower and B-vitamin concentrations were significantly higher among those consuming the folate fortified cereal ($p < 0.001$) (19).

Manilow et al. (20) examined the effects of varying folate levels in ready-to-eat-breakfast cereals in a study similar to Tucker et al (19). Three cereals with equal levels

of B-6, B-12 and varying amounts of folic acid were given to a group of men and women with coronary artery disease (CAD). The subjects were randomly divided and the study was double-blind and placebo-controlled. Results showed increasing intake of folic acid from ready-to-eat-cereal resulted in increase plasma folate and decrease in homocysteine levels (20).

In the Framingham cohort, 65% of the elevated homocysteine cases were found to have inadequate dietary folate or B-12 and B-6, with smaller effects (21). The study also examined total homocysteine in relation to an overall vitamin status. The subjects were divided into five categories; the first category represented individuals in the 70th percentile or better for all three B-vitamins (folate, B-12, and B-6) including intakes from supplements. The fifth category included subjects with intakes below the 30th percentile for B-vitamin intakes; indicating those with the lowest intake of B-vitamins had the highest prevalence of hyperhomocysteinemia at six times that of those in the first category (22).

Major food sources of folate have been reported to include foods such as ready-to-eat cereals, legumes, orange juice, and leafy greens (23). A study examining consumption patterns of 680 white, elderly men and women from the Boston area found four dietary patterns that could be characterized by either high intake of 1) alcohol, 2) milk, cereals, and fruits, 3) bread and poultry, and 4) meat and potatoes (24). Those in the high consumption of meat and potatoes had the lowest folate and B-6 vitamin levels. Those in the group with high intake of milk, cereals, and fruits had the best nutrient profiles including folate and B-vitamins (24).

Homocysteine and cognition

Elias et al. (25), discussing the Framingham Offspring Study, suggested several theories for the relationship between increased age, homocysteine, and cognition. First, is that longer exposure to toxic effects of homocysteine causes increased neural damage. The second theory is that confounding effects caused by deficiency in a vitamin cofactors such as folate or vitamin B-12 exist, indicating elevated homocysteine as more of a marker of poor nutrition than a direct cause for disease (25).

Hyperhomocysteinemia may increase amyloid B-peptide toxicity and increase neuron excitotoxic insults, especially in the hippocampal region (10). Several studies have linked elevated homocysteine levels to increased cognitive decline (6, 26-33). Morris (16) cited several, including Bell et al. (34), Clarke et al. (35), McCaddon et al. (15), and Morris et al. (36), but, one study cited by Morris found no association between homocysteine and cognitive decline.

In vitro studies

The effect of homocysteine on nerve cells has been examined to determine a possible neurotoxic effect. Boot et al. (37) treated neuroepithelial cells with folic acid and homocysteine. The folic acid increased differentiation and outgrowth where addition of homocysteine inhibited neural crest differentiation. This study was focused on modeling the known effects of folic acid and homocysteine in young pregnant women, but do demonstrate interesting effects in regard to homocysteine and folic acid on neural cells and may have implications for normal neural cell function and growth in the brain (37).

Ho et al. (38) treated cortical neurons with homocysteine and discovered a myriad of responses that may link hyperhomocysteinemia to cognitive impairments. The cultures had increased reactive oxygen species, phosphorylated-tau protein, externalized phosphatidyl serine indicating cell death, calcium influx suggesting excitotoxicity, and DNA strand breakage. They also observed decreased levels of SAM, indicating inhibition of methylation reactions (38).

Another study induced homocysteinylation (incorporation of homocysteine into proteins during translation) onto individual serum proteins (39). Treatment appeared to damage proteins (albumin, gamma-globulin, fibrinogen, transferrin, and alpha2-macroglobulin) and inactivated the enzymes methionyl-tRNA synthetase and trypsin (39). This study suggests possible metabolic and biochemical abnormalities which may occur in the presence of excess homocysteine.

Animal studies

It is clear that homocysteine is related in some way to increased risk for chronic diseases like atherosclerosis, stroke, and Alzheimer's but it is whether a risk factor or simply a risk marker for disease is less clear. In one mouse model, apoE deficient mice were fed one of four diets. The first group received a control diet with adequate B-vitamins including folate, B-12 and B-6 (40). The second and third groups were designed to induce B-vitamin deficiencies and hyperhomocysteinemia with or without methionine. And, the fourth diet was a B-vitamin enriched diet. After eight weeks, cognition and memory was measured using the Morris Water Maze test. Inadequate dietary B-vitamin diets did result in decreased plasma levels of B-vitamins, moderately decreased SAM

concentrations in the brain and severely elevated homocysteine in plasma. The mice on these diets were found to have significant cognitive impairments (40). Kruman et al. (11) found that elevated homocysteine increased hippocampal neurons likelihood to excitotoxic and oxidative injury in rat studies.

Another mouse model found B-vitamin deprivation (for 45 and 60 days) to be associated with hyperhomocysteinemia and a decrease of SAM/SAH ratio (41). This was accompanied by an increase in amyloid-β deposition and cognitive impairments in maze tasks (41). Bernardo et al. (42) found that hyperhomocysteinemia induced diets were associated with decreased cognition and increased neurodegeneration in mice that over-produce APP.

Other animal studies have explored the effects of one-carbon metabolism gene mutations or deletions; ultimately leading to elevated homocysteine. Chen et al. created a mouse model with the MTHFR gene deleted (43). They found the associated metabolic outcomes of the mutation varied based on tissue location. SAM concentrations remained unchanged in the liver but were significantly decreased in the brain. SAH concentrations were significantly elevated in both locations. Interestingly, methylation was only significantly impaired in the brain (43). Application of these results to humans is cautioned because the level of elevated homocysteine in this model is much higher than would be seen in a B-vitamin deficient or hyperhomocysteinemia model in humans. The study does suggest severely elevated levels may have large impacts on brain biochemistry and metabolism (43).

Cross-sectional studies

The famous Nun Study reported on sixty nun's from convents in the US (midwest, east, and southern areas) with the objective of comparing brain infarcts, areas of cell death, and clinical manifestation of AD (44). The nuns came from similar environment, had similar nutritional habits, and ranged in ages from 75-100. The brains were dissected and examined post mortem for vascular changes and damage and increased severity of dementia was found in nuns with significant brain infarcts. Half the nuns (45) had neuropathological lesions of AD and folate was the only nutritional factor examined out of 18 that was negatively correlated with the atrophy (13, 46).

A case-control study involving 55 non-cognitively impaired, 81 mildly impaired, and 92 diagnosed AD or vascular dementia subjects (average age ~75) explored the relationship of homocysteine, B-vitamins and cognition (21). Participants came from a Memory Clinic of the Ospedale della Beata Vergine, Mendrisio, Switzerland. Quadri et al. (21) included a mildly impaired group for the purpose of exploring the question of whether elevated homocysteine exists prior to AD diagnosis suggesting its role as a risk factor or if it has only been observed as a marker or as a cause of vascular damage. This study found those with the lowest tertile of serum folate had higher odds (OR 3.1) of dementia and cognitive impairment, and there was an inverse association between folate and homocysteine. Results from this study indicated that folate deficiency and hyperhomocysteinemia may be present prior to the onset of dementia. This study found no relationship between vitamin B-12 and cognitive decline but examined serum levels of cyanocobalamin which has been noted as a less indicative marker of B-12 status than holotranscobalamin or methylmalonic acid (MMA) (21).

In 2002, Duthie et al. (47) recruited survivors of two waves of Scottish Mental Surveys (years 1932 and 1947). From the 186 and 148 survivors gathered from each wave, they observed positive associations between serum folate, B-12 and scores from the Mini Mental State Examination (MMSE). Negative associations were seen between both vitamins and homocysteine levels. In addition, homocysteine was negatively associated with other tests of cognition including Raven's Progressive Matrices (RPM), digit symbol (DS) subtest, and block design (BD) subtest (47).

Lindeman et al. (48) analyzed 883 Hispanic and non-hispanic whites males and females, ages 65 or older, from Bernalillo County, New Mexico to examine relationships of vitamin serum markers and cognitive decline. Serum folate, vitamin B-12 and vitamin C status were measured and compared to cognitive function. Usual dietary intake was estimated using a Food Frequency Questionnaire (FFQ). Cognitve function was assessed using a mixture of tests including the MMSE, WAIS-R Digits Forward, Fuld Object Memory Evaluation, clock drawing, two Color Trial Making Tests. There was a significant association between dietary intake of folate and serum folate measures ($p < 0.001$) and dietary B-12 and serum B-12 ($p < 0.05$). The strongest associations were between folate status and various measures of cognition ($p = 0.002$). Lower folate (<11.1 nmol/L) status was associated with lower cognitive scores compared with normal folate status. Serum B-12 was not associated with cognitive score (48).

In the Normative Aging Study, Riggs et al. (31) reported decreased folate and vitamin B-12 levels to be related to lower spatial copying skills in 70 men ages 54-81 from the Boston area. Cognition was assessed using the MMSE, verbal fluency and constructional praxis adapted from the revised Wechsler Adult Intelligence Scale and the

CERAD batteries. Low B-12 ($p = 0.04$), folate ($p = 0.003$), and elevated homocysteine ($p = 0.0009$) were all related to poor spatial copying skills. They also found elevated homocysteine (>12.6 µmol/L) to be a stronger predictor than folate and B-12 for spatial copying skill impairment (31).

In a recently published article, Morris et al. (10) reported serum folate (<59 nmol/L) and low B-12 status (serum vitamin B-12 concentration <148 pmol/L or a serum methylmalonic acid concentration >210 nmol/L) to be related to increased risk for anemia (odds ratio (OR): 2.7; 95% CI: 1.7, 4.2) and cognitive inpairment (OR: 2.5; 95% CI: 1.6, 3.8) compared to those with normal B-12 status. In a comparison of those with low B-12 and high serum folate (>59 nmol/L) the odds of anemia (OR: 3.1; 95% CI: 1.5, 6.6) and cognitive impairment (OR: 2.6; 95% CI: 1.1, 6.1) was increased. This supports the theory that high folate masking may be masking a B-12 deficiency. The analysis included 1459 men and women ages 60 and older from the 1999-2002 US National Health and Nutrition Survey. Cognition was assessed using the Digit Symbol Coding test and the MMSE (10).

Prospective studies

Homocysteine levels were first found to be related to arteriosclerosis in 1969 by McCully who observed hyperhomocysteinaemia and arterial damage in two children (21). Since then, hyperhomocysteinaemia has been associated with increased risk for heart disease, stroke, and cognitive function decline in observational and clinical trials (19, 49). Factors associated with hyperhomocysteinaemia include genetic mutations, vitamin deficiencies, renal and other diseases, drugs, and increased age (45). The

Framingham Heart Study found that 29% (n=885) of the original free living cohort, age 40-83 years old, had hyperhomocysteinaemia (homocysteine concentration >14 umol/L) (18, 19). Suggesting that elevated homocysteine is common among the elderly groups.

Studies examining cognitive decline and serum markers

The MacArthur Studies of Successful Aging included 499 high-functioning persons ages 70-79 from Durham, North Carolina; East Boston, Massachusetts; and New Haven, Connecticut (28). Cognitive decline was assessed using a battery of cognitive tests including the Boston Naming test, perception and reproduction of spatial associations, Delayed Recognition Span Test, Wechsler Adult Intelligence Scale-Revised, and abstract concept formation. Researchers found persons in the lowest quartile of plasma folate (1.17 to 3.15 ng/mL) had 1.6 times the risk for cognitive decline over 7 years (28).

The Leiden 85 plus Study is a cohort of 599 elderly subjects from the Netherlands, 85 years of age and older with a mean four year follow up (50). Mooijaart et al. (50) investigated the relationships between serum levels of folate, B-12, homocysteine and rate of cognitive decline. A series of cognitive tests were used to assess decline including the MMSE, Stroop test, a letter digit coding test, and word recall test. No association between any of the serum markers and cognitive decline was identified. One limitation put forward by the authors was the use of serum vitamin B-12 concentrations as assessment for B-12 status, which is considered a poorer marker of metabolic vitamin B-12 deficiency (50).

Another study involving a larger span of ages (30-80 years) in 144 men and women from the Netherlands for 6 years examined the relationship between plasma homocysteine, B-vitamins and cognitive decline (6). Cognition was assessed using the Letter-Digit coding test, Stroop test, Word Learning Test total and delayed recall. They found no relationship between serum levels of homocysteine, B-12 and folic acid and rate of decline at any measurement point over the 6 years (6).

Studies examining cognitive decline and dietary intake

The Chicago Health and Aging Project (CHAP) study involved 3718 AD free participants older than 65 years from the Chicago area (7). Usual dietary intakes were estimated using a 139-item FFQ. Cognition was assessed using the East Boston Tests of immediate and delayed recall, the MMSE, and the Symbol Digit Modalities Test. In a 6-year follow-up, Morris et al. (10) found that those in the top quintile of total folate intake (median 742 µg per day) had more than twice the rate of cognitive decline compared to the lowest quintile (median, 186 µg per day). The top food folate quintile (median, 382 µg per day) was associated with faster decline in an adjusted model. High total B-12 intake was associated with slower cognitive decline in the oldest participants (10).

Studies examining cognitive decline, serum markers and dietary intake

Only one study to date has examined cognitive decline, serum markers and dietary intake (51). In 2005, Tucker et al. (51) published a paper on a larger group from the Normative Aging Study that included 321 men age 50-85 years old that were followed for over 3 years. The objective of the study was to examine associations between dietary and serum B-vitamin measures with cognition. Cognition was assessed

using the MMSE, verbal fluency and constructional praxis adapted from the revised Wechsler Adult Intelligence Scale and the CERAD batteries. Diet was assessed using a 126-item FFQ. Decreased spatial copying was significantly associated with low plasma measures of all three vitamins (folate, B-12, and B-6), elevated homocysteine, and dietary intakes of each vitamin. They found higher dietary folate (<339µg/d) to be protective of verbal fluency decline and hyperhomocysteinaemia (>11nmol/L) related to a decline in recall memory ($p < 0.05$) (51).

Studies examining incident dementia/AD and serum markers

The Conselice Study of Brain Aging (CSBA) is a prospective population-based survey of 816 dementia-free Italians with a mean age of 74 years (52). Dementia was diagnosed using the Diagnostic and Statistical Manual of Mental Disorders-IV clinical criteria. Cox proportional hazards regression modeling accounting for covariates such as time, sex, genotype, education, etc. was used to determine relationships between homocysteine and incident AD. An association between baseline homocysteine, risk of newly diagnosed dementia and AD was examined after a 4-year period (1999-2000 to 2003-2004). The results suggested that both elevated homocysteine (>15µmol/L) (HR:2.08 95% CI: 1.31, 3.30 for dementia and HR: 2.11; 95% CI: 1.19, 3.76 for AD) and low serum folate (<11.8 nmol/L) (HR: 1.87; 95% CI: 1.21, 2.89 and HR: 1.98; 95% CI: 1.15, 3.40) were independently related to risk for both dementia and incidence of AD. This study did not find an association with B-12 and incident dementia or AD (30, 52). This study adjusted for serum creatinine, but did not use the holotranscobalamin or MMA measurement, better measures of B-12.

Luchsinger et al. (53) also examined homocysteine and risk for AD in the Washington Heights-Inwood Columbia Aging Project (WHICAP). A group of 909 elderly participants, average age 77.2 were randomly chosen from a group who had received Medicare assistance and followed for about 1.5 years. The results showed no relationships between incidence of AD and high serum homocysteine, nor any association between elevated homocysteine and cognitive decline over time ($p = 0.31$). The relationships were significantly confounded by age. One limitation disclosed by the authors was a lack of power to detect modest associations (53).

A study by Wang et al. (54) involved 370 non-demented participants from the Kungsholmen Project. Participants were men and women from Stockholm, Sweden, ages 75 years, plus and were followed for three years. The objective of the study was to examine serum folate and B-12 in relation to incident AD. They did find significant, nearly doubled risk for AD, among participants who were deficient in either folate (<10 nmol/L) or vitamin B-12 (<150 pmol/L). The relative risk (RR) among those with low folate or B-12 for AD was 1.4 (95% CI: 1.2, 3.5) (6).

Though other studies have not reported similar findings when examining serum vitamin levels and incident AD, if the results of Wang et al. (54) are to be accepted, further consideration for whether dietary intakes are actually reflected in serum levels and how closely they are reflected is required. Several studies have shown positive associations between dietary or supplemental intake of B-vitamins and associated serum biomarkers, including Tucker et al. ($P < 0.0001$) (19), Manilow et al. ($P = 0.045$) (20), Selhub ($P < 0.01$) (22).

An earlier study (2002) by Seshadri et al. (33) on the Framingham offspring cohorts found hyperhomocysteinaemia present in subjects pre-diagnosis of AD. The analysis included 1092 dementia-free participants with a mean age of 76 years. Participants were followed for eight years. Those with hyperhomocysteinaemia had increased risk for both incident AD and dementia (RR 1.8 {95% CI: 1.3, 2.5} and 1.4 {95% CI: 1.1, 1.9} respectively) (33).

Studies examining incident dementia/AD and dietary intake of B-vitamins

A prospective cohort study among the participants of the (WHICAP) studied 965 dementia-free elders, 65 years and older from the northern Manhattan area (5). Luchsinger et al. (5) examined relationships between dietary intake of folate, B-12 and B-6 and incident AD. A 61-item FFQ was used to assess nutrient intake. Dementia and AD diagnoses were made using the Diagnostic and Statistical Manual of Mental Disorders. After six years, 192 participants developed AD. The highest quartile of folate intake (>489.7 µg) was related to lower risk for AD (HR: 0.5; 95% CI: 0.3, 0.9); there was no associations between B-12, B-6 and AD (5, 6).

Morris et al. (55) observed no association between incident AD and dietary intake of B-vitamins among participants of the CHAP. The CHAP study, previously mentioned, is a prospective study which involved 1,041 initially AD free biracial elders, age 65 plus, from the Chicago community. Dietary assessment was made using a 139-item FFQ. AD diagnoses were made based on tests of the Consortium Established for Research on Alzheimer's Disease (CERAD). Limitations of this study include many of the dietary assessments were collected after the baseline interviews and no serum markers were

collected. The CHAP study did identify a relationship between dietary intake of B-vitamins and protective factors for AD such as younger age, white race, higher education, etc. (55).

Another study, by Corrada et al. (56), with a sample of 579 non-demented elderly volunteers ages 49-93 from the Baltimore Longitudinal Study of Aging, found higher total folate intake was associated with lower risk for AD (RR: 0.41; 95% CI: 0.22 0.76) (6). Dementia was diagnosed using the *Diagnostic and Statistical Manual of Mental Disorders*, third edition and AD diagnosis made using criteria from the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders. Diet was assessed from seven day dietary records kept by participants. The subjects were followed for an average of 9.3 years. No association was found between total intake of vitamin B-12 and AD (6).

Studies examining cognitive decline, incident dementia/AD and serum markers

Researchers from the Sacramento Area Latino Study on Aging have also recently published a paper on this topic (27). The study included a cohort of 1170 Mexican Americans ages 60-101 who were followed over 4-5 years. Haan et al. (27) used proportional hazards modeling to examine associations between serum markers (RBC folate, plasma B-12, homocysteine, and serum creatinine) and incident dementia and cognitive impairment without dementia (CIND) assessed using the MMSE and Verbal Episodic Memory test. They found hyperhomocysteinaemia (>13 µmol/L) to be an independent risk factor for dementia (HR: 1.58; 95% CI: 1.88, 2.83). High vitamin B-12 (>498 pg/mL), but not folate, was associated with low risk of homocysteine related

dementia (dementia related to elevated levels of homocysteine) or CIND (HR: 2.5; 95% CI: 1.31, 4.54). This study did adjust for serum creatinine levels but did not use the holotranscobalamin or MMA measure. The authors note that though other studies have failed to look for or find the same relationship of B-12 and dementia, the relationship has been seen enough that further investigation should be done. Morris et al. (7), Tucker et al. (51), Wang et al. (54), and Hann et al. (27) have noted associations between B-12 and dementia.

Possible explanations for conflicting results

Though several studies have shown a negative relation between B-12 status and homocysteine levels, when Refsum and Smith (57) examine the relationship measuring the active form of B-12, holotranscobalamin, participants with AD were found with significantly lower levels of cobalamin. Since 80% of total cobalamin is bound to haptocorrin and is not available to the cells, holotranscobalamin is a better indicator of B-12 status in relation to deficiency effects (57). Another study using the holotranscobalamin measure involving 1000 elderly from the UK found independent relationships between cognitive decline and low serum holotranscobalamin with high homocysteine. They noted that relationships were significantly higher with that measure compared to that of vitamin B-12 (58).

A case-control study mentioned earlier by Quadri et al. (21) stated that one reason that may account for other studies finding differing results (relationship between homocysteine, B-vitamins and dementia) is the fact that many studies did not adjust for serum creatinine when looking at homocysteine. Quadri et al. (21) mentioned that two

other studies which did adjust had similar results to their study. Adjusting for serum creatinine is important due to the highly correlated relationship between serum creatinine and homocysteine (and other B-12 markers). Increased creatinine by 100 µmol/L doubled levels of MMA, holotranscobalamin, and homocysteine in one study (59). Creatinine may be elevated for a number of other reasons unrelated to vitamin deficiency.

TABLE 2.1
Summary of prospective studies

Authors	Design	Subjects	Methods	Results
Studies Examining Cognitive Decline and Serum Markers				
Kado et al. 2005 (28)	Cohort, serum homocysteine (hcy), B-vitamins related to cognitive scores in high-functioning elders, mean follow-up of 7 years	N = 499 men and women age 70-79, from NC, MA, CN involved in the MacArthur Studies of Successful Aging	Subjects administered Boston Naming test, perception and reproduction of spatial associations, Delayed Recognition Span Test, Wechsler Adult Intelligence Scale-Revised, and abstract concept formation	Low folate status increased risk for cognitive decline
Mooijaart et al. 2005 (50)	Cohort, serum hcy, folic acid, vit B12 related to cognitive decline, mean follow-up of 4 years	N = 599 men and women from the Leidan 85-Plus Study, from the Netherlands	Subjects assessed using *MMSE, Stroop test, a letter digit coding test, a word recall test	No associations
Teunissen et al. 2003 (60)	Cohort, serum hcy, folate, vit B-12 associations with cognitive decline, mean follow-up of 6 years	N = 144 men and women, ages 30-80, from the Netherlands	Subjects administered Letter-Digit coding test, Stroop test, Word Learning Test total, and delayed recall	no associations

TABLE 2.1, CONTINUED
Summary of prospective studies

Authors	Design	Subjects	Methods	Results
Studies Examining Cognitive Decline and Serum Markers				
Ravaglia et al. 2005 (52)	Cohort, plasma hcy, folate, and vit B-12 to Incident dementia and AD, with mean follow-up of 4 years	N = 816 dementia-free, Italians, mean age 74 from the Conselice Study of Brain Aging (CSBA)	Subjects assessed by qualified clinicians	Elevated hcy and low serum folate were related to increased risk for dementia and AD. No associations were found between B-12 and incident dementia/AD
Luchsinger and Mayenx 2004 (61)	Cohort, plasma hcy associations with incident AD, mean follow-up 1.5 years	N = 909 men and women mean age 77.2 from the Washington Heights Inwood Columbia Aging Project (WHICAP)	Subjects assessed by qualified clinicians	No associations
Wang et al. 2001 (54)	Cohort, plasma vit B-12 and folate to incident AD, mean follow-up 3 years	N = 370, men and women older than 75 from Stockholm, Sweden participating in the Kungsholmen Project	Subjects assessed by qualified clinicians	Low levels of B-12 or folate were independently related to double the risk for incident AD
Seshadri et al. 2002 (33)	Cohort, serum hcy to incident dementia and AD, mean follow-up 8 years	N = 1092 dementia free participants with a mean age of 76 from the Framingham cohort	Subjects assessed by qualified clinicians	Elevated hcy related to increased risk for both incident AD and dementia
Studies Examining Incident Dementia/AD and Dietary Intake				
Luchsinger et al. 2007 (5)	Cohort, dietary intake of folate, vit B-12 and vit B-6 with incident AD, mean follow-up of six years	N = 965 men and women 65 years and older from the Washington Heights Inwood Columbia Aging Project (WHICAP)	Subjects assessed by qualified clinicians	Highest intake of folate was related to lower risk for AD. No associations observed between vit B-12, vit B-6 and AD.

TABLE 2.1, CONTINUED
Summary of prospective studies

Authors	Design	Subjects	Methods	Results
Corrado et al. 2005 (56)	Cohort, dietary intake of folate, vit B-12 to incident AD, mean follow-up average of 9.3 years	N = 579 men and women ages 49-93 from the Baltimore Longitudinal Study of Aging	Subjects assessed by qualified clinicians	No associations found with B-12, higher folate and B-6 were independently associated with decreased risk. Folate was the only associated vitamin in an model with all three vitamins
Studies Examining Cognitive Decline, Incident Dementia/AD and Serum Markers				
Hann et al. 2007 (27)	Cohort, serum hcy, folate, vit B-12 to incident dementia and cognitive decline, follow-up of 4-5 years	N = 1170 Mexican American men and women ages 60-101 from the Sacramento Area Latino Study on Aging	Assessed by qualified clinicians and using the MMSE and Verbal Episodic Memory test.	High hcy was associated with increased risk for dementia. High B-12 was associated with low risk for homocysteine related dementia or cognitive impairment

*Mini-Mental State Examination

Human intervention trials

Studies examining folic acid only

In 2007, a paper published on the FACIT (folic acid and carotid intimamedia thickness trial) reported possible protective effects in cognitive performance from supplementation of folic acid. This was a large randomized, double-blind, control trial that had 818 men and women with elevated homocysteine and normal B-12 markers, ages 50-70, divided into placebo and supplemental groups. The supplemental group received 800 µg of folic acid per day (twice the recommended amount) for three years. They had 576% higher serum folate and 26% lower homocysteine levels after the three years and

significantly better changes in memory (measured by the Verbal Learning Test) compared to the placebo group (26).

Fioravanti et al. (62) reported cognitive function improvement in 30 participants with abnormal cognitive decline and low folate levels (3 ng/ml or less) supplemented with folic acid for 60 days. The improvement was positively correlated with degree of folate deficiency at baseline (62).

Studies examining folic acid and other B-vitamins

Another clinical study found supplementation with neither vitamin B-12 nor a combination of B-12 and folic acid supplementation provided any improvement in cognitive function (63). Participants were 195 elderly, aged 70 plus, from different parts of the Netherlands, with mild vitamin B-12 deficiency, defined by either serum B-12 between 100-200 pmol/L or serum B-12 between 200-300 pmol/L, MMA greater than 0.32 µmol/L, and serum creatinine greater than 120 µmol/L. Groups were randomized to receive 1000 µg B-12 plus 400 µg folic acid, 1000 µg B-12 alone, or placebo for 24 weeks. Cognition was assessed using the Mini-Mental State Examination (MMSE), and Clinical Dementia Rating (CDR) (63).

McMahon et al. (64) found B-vitamins had no effect on cognition in a supplement trial of 1000 µg of folic acid, 500 µg of vitamin B-12 and 10 mg of vitamin B-6. Participants included 276 healthy elderly men and women, ages 65 and older, with homocysteine levels greater than 13 µmol/L. After two years of intervention, homocysteine levels were significantly lower in controls but there was no difference in cognition between the groups (64).

The Vital Trial Collaborative group randomized 149 individuals at high risk for dementia into three treatment groups of aspirin, a variety of vitamin supplements (folic acid, vitamin B-12, vitamin E, and vitamin C), and placebo. The subjects were assessed to be at increased risk for dementia using MMSE and Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog). B-vitamins had no effect on cognitive function, but did lower homocysteine by 30% (6).

Two older trials found some positive effects from B-vitamin supplementation. Bryan et al. (65) saw the improvement in cognitive function (assessed by a battery of different cognitive tests) using short-term supplementation in 211 healthy women of various ages for 60 days. Participants received 750 µg folic acid, 15 µg vitamin B-12, and 75 mg of vitamin B-6 or placebo.

In a small supplementation trial involving 33 subjects exhibiting with mild-moderate dementia and a mean age of 78.4, researchers examined the effects of supplementing with 500 µg folic acid and 1 mg of vitamin B-12 a day for 2 months on homocysteine and cognition using the MMSE. Blood samples were collected before and after supplementation. Five participants who were deemed severely demented and unable to follow study procedures were excluded. Of the remaining 28 participants, two groups were formed based on baseline homocysteine levels cut at 19.9 µmol/L. Subjects with mild-moderate dementia and elevated homocysteine at baseline did benefit from the supplementation regime and exhibited higher MMSE scores post treatment. Those without elevated homocysteine did not improve cognitively (29).

The relationship between B-vitamins, homocysteine, and dementia is not universally accepted. Morris et al. (66) strongly state that current research does not

validate associations between B-vitamins and AD with the exception of B-12 deficiency and cognitive decline. B-12 deficiency has been widely accepted to be characterized by cognitive and psychiatric disturbances due to B-12's role in metabolizing methylmalonic acid (4, 15, 66).

TABLE 2.2
Summary of human intervention trials

Authors	Design	Subjects	Methods	Results
Studies Examining Folic Acid Only				
Durga et al. 2007 (26)	Randomized clinical trial, effects of folic acid supplementation on cognitive performance	N = 818, men and women ages 50-70, with elvated homocysteine (hcy) from the Folic acid and carotid intimamedia thickness trial (FACIT)	Supplemental groups receive 800 µg of folic acid per day for three years. Cognition was measured using the Verbal Learning Test	Supplemental group had 576% higher serum folate and 26% lower hcy levels. They also had significantly better changes in memory
Fioravanti et al. 1997 (62)	Randomized clinical trial, effect of folic acid supplementation in people with abnormal cognitive decline and low serum folate	N = 30 participants with abnormal cognitive decline and low serum folate	Participants received 15 mg of folic acid for 60 days. Cognition was assessed using Randt memory test, acquisition and recall, delayed recall, memory index, encoding factor, cognitive efficiency, and attention efficiency	Cognitive improvement was seen in the group, degree of improvement was negatively correlated to degree of baseline folate deficiency
Studies Examining Folic Acid and Other B-vitamins				
McMahon et al. 2006 (64)	Randomized clinical trial, effects of a variety of supplements (folic acid, vit B-12, vit B-6) on cognitive decline and incident AD	N = 276 healthy elders age 65 years and older with elevated hcy levels.	Groups were randomized to receive 1000 µg folic acid, 500 µg of B-12, and 10 mg of B-6 for two years	There was a significant decrease in hcy levels over controls but no difference in cognition between the groups

TABLE 2.2, CONTINUED

Summary of human intervention trials

Authors	Design	Subjects	Methods	Results
Clarke et al. 2003 (67)	Randomized clinical trial, effect of supplementation with folic acid and vit B-12 versus placebo on cognitive function	N = 149, high risk of dementia men and women from the Vital Trial Collaborative group	Groups were randomized to receive 2 mg folic acid, 1 mg vit B-12 or placebo. Cognition was assessed using the MMSE and the Alzheimer's Disease Assessment Scale Cognitive subscale (ADAS-Cog)	Supplemented group had hcy levels decreased by 30%, they saw no difference in cognitive function
Bryan et al. 2002 (65)	Randomized clinical trial, effect of B-vitamin supplementation on cognitive decline	N = 211 healthy women, from various ages	Groups were randomized to receive 750 µg folic acid, 15 µg vit B-12, and 75 mg of vit B-6 or placebo. Cognition was assessed using 18 different cognitive tests	Supplemented group had improvement in cognitive function
Nilsson et al. 2001 (29)	Randomized clinical trial, effect of supplementing with 500 µg of folic acid and 1 mg of vit B-12 on homocysteine and cognition	N = 33 subjects with mild-moderate dementia, mean age 78.4, refer to the Psychogeriatric department of the Lund University Hospital	Subjects received 500 µg of folic acid and 1 mg of vit B-12 for 2 months. Cognitive assessment was done using the MMSE	Those with elevated homocysteine at baseline did benefit from supplementation as seen by increase MMSE scores

*Mini-Mental State Examination

Meta-analyses of supplemental trials

A meta-analysis of randomized trials done by the Homocysteine Lowering Trialists' Collaboration included 2596 subjects from 25 controlled randomized studies published from 1993-2003. They found supplementation with 0.5 to 5 mg of folic acid a day for a mean of eight weeks lowered homocysteine levels by 25% among individuals

with normal folate status (12 nmol/L). The addition of .02-1 mg of B-12 showed further reduction by 7%. B-6 and betaine were less effective at reducing homocysteine (45). The timing of this meta-analysis is important because most trials were done prior to fortification of the food supply with folic acid. Since fortification the results in the studies examine by the meta-analysis may be less robust due to the decline in folate deficiency prevalence.

Shah et al. (68) examined results from studies on homocysteine, cognitive functioning, hormone levels, and exogenous estrogen use in women published from 1993 to 2005. Elevated homocysteine was regularly associated with cognitive decline. They concluded that there is not sufficient evidence to suggest that homocysteine levels are linked to cognitive decline, and that dietary supplementation with B-vitamins assists with homocysteine level control without evidence that this change has any effect on cognition (68).

Ellison et al. (69) identified six studies meeting criteria to analyze relationships between serum folate, B-12, homocysteine and cognitive impairment. Three case-control studies and three cohort studies with subjects over 60 years old were included. The majority of the studies used the MMSE to assess cognition. Total homocysteine was found to be associated with cognitive scores; folate and B-12 status was not (69).

Meta-analyses of cardiovascular-health related trials

Efficacy of folic acid supplementation on cardiovascular health related outcomes has been more extensively studies than trials on cognitive outcomes. Results from meta-analyses of these trials were also inconsistent.

de Bree et al. (70) conducted a meta-analysis on folic acid trials and vascular reactivity spanning from 1966-2005. Only fourteen of over 150 trials met exclusion criteria. Folic acid doses ranged from 600-5000 µg/d. Folic acid appeared to increase endothelial function 1.08 (95% CI: 0.57,1.59; $P = 0.0005$). This may have implications for reducing heart disease (70). Data from this study include trials from both pre- and post-fortification.

A recent meta-analysis of folic acid, homocysteine and cardiovascular disease by Wald et al. (71) examined cohort studies and randomized trials. Results from sixteen cohort studies show comparable relationships between decreases in homocysteine and risk of heart disease. Among the seven randomized trials short term benefits were observed but Wald et al. state statistical power is lacking preventing conclusiveness of these results (71).

Bazzano et al. (72) investigated studies from 1966 to 2006 on folic acid supplementation and risk for cardiovascular diseases. Studies that met the criteria included a total of 16,958 participants with vascular disease. The relative risks for folic acid supplemented participants with cardiovascular disease, coronary heart disease, stroke, and all-cause mortality were as follows: 1.04 (0.92-1.17) , 0.86 (0.71-1.04), and 0.96 (0.88-1.04) respectively. The authors concluded there is not sufficient evidence suggesting folic acid supplementation reduces risk for vascular diseases (72) .

Supplements versus dietary sources

Supplementation with B-vitamins while examining cognitive function has been explored with varying results. The inconsistencies found between these studies are vastly

due to the differences in methods and procedures. Supplementation trials summarized by a task force on nutrition and cognitive decline reported 50% of reviewed trials saw improvements in cognitive function and B-vitamin supplementation (6). The other half of the trials saw no improvements (6). Selhub et al. (1) mentions results from studies by Lendenbaum et al. and Martin et al. which report improvement in cognitive recovery in B-12 deficient subjects with supplementation of the vitamin, suggesting poor vitamin status may partially be the cause of cognitive impairment seen in some elderly (1).

Studies that have examined dietary intake of B-vitamins in relation to cognitive health also have varying results. Luchsinger et al. (5) did observe decrease risk for incident AD/dementia in participants who consumed high amounts of total folate (food plus supplement), but the relationship was not significant with high intake of folate from food only. Morris et al. (55) and Corrado et al. (56) did not see a reduction of risk for incident AD. The conflicting results are not limited to positive and no associations. Morris et al. (7) reported that high dietary folate intake was related to faster cognitive decline in the CHAP group.

The bioavailability of supplemental and fortified folic acid sources appears to be higher than that of folate found naturally in food (73). Actual assessment of absorption of folate and other B-vitamins is very difficult. There are over 150 different forms of folate and common conditions such as gastritis are known to inhibit B-12 absorption. One source noted that 50-95% of folate in food is estimated to be lost in storage, preparation and manufacturing (74). Based on the current literature there is not enough evidence to suggest supplementation with B-vitamins in persons with adequate serum

levels for improvement in cognitive impairment. Supplementation in deficient persons is warranted and may provide cognitive improvements.

CONCLUSION

As stated by Morris et al. (55) despite the extensive amount of attention given to folate, other B-vitamins and homocysteine in the area of cognitive health among the elderly, there is not enough evidence that a causal relationship between B-vitamins and cognitive health exists (55). It is also evident that the results from these many studies are often contradictory and inconsistent because study designs and methods vary greatly. Among clinical trials duration of supplementation, control of diet, dosage, combination of vitamins used, and types of assays used to assess nutriture vary greatly. Measures of cognitive status also vary greatly. It may be possible that improvements may be seen in only certain areas of cognitive health, which may be detected by only certain tests. There are many limitations to assessing usual dietary intake of B-vitamins. Dietary assessment tools such as the FFQ, commonly used provide less precise estimates of usual intake than more burdensome multiple day recall a recording methods. In addition, bioavailability between forms of B-vitamins like folic acid and B-12 vary. Finally, amongst the elderly, common medical conditions like gastritis are known to alter absorption of B-vitamins.

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CHAPTER 3

DIETARY FOLATE, OTHER B-VITAMINS AND INCIDENT ALZHEIMER'S DISEASE: THE CACHE COUNTY MEMORY, HEALTH, AND AGING STUDY[1]

ABSTRACT

Background B-vitamins, especially folate, are of interest in regards to cognition due to their roles in one-carbon metabolism.

Objective To examine associations between dietary and supplemental folate, vitamin B-12 and vitamin B-6 and incident Alzheimer's disease (AD) among elderly men and women.

Design This study analyzed data collected from the Cache County Memory, Health and Aging Study, a longitudinal study of men and women elders 65 years and older who were residents of Cache County, Utah. Multistage clinical assessment procedures were used to identify incident cases of AD. Dietary data was collected using a 142 item Food Frequency Questionnaire. Cox Proportional Hazards modeling was used to determine hazard ratios across quintile intake of micronutrients. The models controlled for the effects sex, education, physical activity, BMI, energy intake, and other covariates.

Results 202 participants were diagnosed with incident AD during follow-up (1995-2004). There were no observed differences in risk of AD or dementia by increasing quintiles of total intake of folate, vitamin B-12, and vitamin B-6. Users of supplemental folic acid were more likely to have healthy diet and lifestyle factors although no association was observed those between using <400 µg/d and dementia or AD.

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Conclusion Dietary intake of B-vitamins from food and supplemental sources, individually or combined appear unrelated to incidence of dementia and AD. Further studies examining associations between dietary intakes, biomarkers of status and cognitive endpoints are warranted.

INTRODUCTION

The prevalence of Alzheimer's disease (AD) is expected to more than double in the next fifty years with the aging of the baby boomers (1, 2). In 2004, it was reported that 2% of the population of industrial countries population were affected by AD (2). AD is currently the third most costly disease in the US coming in behind cancer and heart disease (3). In 2003, AD increased the cost of health care by 80-100 billion dollars (3).

AD is undoubtedly an age-related disease, but risk for AD, the severity, and rate of decline are influenced by genetic as well as modifiable environmental factors including diet (3, 4). Although many dietary factors likely play a role in cognition, relationships between folate, vitamin B-12, and vitamin B-6 have recently gained interest (5).

Mechanistically there are several explanations for the possible relationship between B-vitamins and AD. Folate, vitamin B-12 and vitamin B-6 are key methyl carriers and cofactors of one-carbon metabolism (6). Deficiencies of folate and B-vitamins are known to be accompanied by increased levels of homocysteine, an intermediate of one-carbon metabolism (7). In vitro studies, homocysteine has been identified as a possible neurotoxin, as it may increase oxidative stress and damage leading to neuronal cell death (4, 8). During the last ten years research involving

increased risk for heart disease and hyperhomocysteineamia has been widely published (9-12). A similar relationship between homocysteine and cognition is also being extensively investigated (5, 7, 8, 13-24).

Several mechanisms of disrupted one-carbon metabolism may link risk for AD to B-vitamins, including decreased concentrations of S-adenosyl-methionine (SAM). SAM is the primary methyl carrier for methylation reactions in the brain and is formed from methionine. Methionine comes from the diet and is formed from homocysteine which requires adequate folate and vitamin B-12 (25). Finally, folate in particular may affect cognitive health due to its essential role in nucleotide formation. Deficiency may alter normal DNA/RNA synthesis, which may impact neuron formation and function (25).

The elderly population is not only at increased risk for AD but also have higher rates of B-12 deficiency (26). B-12 deficiency is characterized by macrocytic anemia, neuropathy, and irreversible neurologic damage in severe cases. B-12 is a cofactor for two enzyme reactions. In addition to B-12's role in one-carbon metabolism, as a part of the methionine synthetase, B-12 also acts as a cofactor to the reaction converting methylmalonic acid to succinyl-CoA (27). Absorption of vitamin B-12 is dependent on the environment of the gastrointestinal tract and is often impaired among elderly with gastritis or drugs that decrease gastric acid like antacids (28). Vitamin B-12 is naturally only found in animal products. The Framingham study reported that 24-37% of the cohort could be diagnosed with gastritis depending on diagnoses criteria used (6). About 20% of the cohort was folate deficient, 20-25% were B-12 deficient, and about 20% were B-6 deficient in the early 1990's. One in twenty elders over 65 and one in ten over 75 were found to be B-12 deficient in a population from the United Kingdom (26). Serum

levels of B-12 are often used to assess status, but inconsistency in the use of biomarkers to diagnose vitamin B-12 deficiency may be contributing to underestimates in actual B-12 deficiency prevalence (29).

Since the mandate to fortify cereal and grain products in the US with folic acid, a decreased prevalence of folate deficiency has been observed (30). With the increase of folate intake and status, many have voiced concern of a potential effect of folate masking deficiencies of other B-vitamins, primarily vitamin B-12. The "masking effect" is based on the observation that a vitamin B-12 deficiency diagnosis becomes more difficult as the main symptom, macrocytic anemia, it often alleviated with folic acid supplementation and simultaneously 'masks' the underlying B-12 deficiency which may lead to irreversible cognitive impairments depending upon the length and severity of the deficiency (31). A study on data from NHANES observed participants with high serum folate in the presence of low vitamin B12 status had increased risk for both anemia and cognitive decline (32).

Research on this topic is inconclusive as many studies report conflicting results (5). Among prospective studies high levels of folate intake, defined by intake more than 557 µg/d has been identified as both harmful and protective in relation to cognitive decline and incidence of AD (24, 33). Other studies have shown no relationship between folate, B-vitamins and AD (34). The objective of this study is to examine relationships between folate, vitamin B-12, vitamin B-6 and incident AD, as well as to consider mediating effects of varied combinations of adequacy and deficiency of B-vitamins, among elderly participants of the Cache County Memory, Health, and Aging study with over nine years of follow-up.

SUBJECTS AND METHODS

Subjects

The Cache County Memory Study is a population based prospective study of men and women who were living in Cache County, Utah and 65 years or older at the baseline assessment in 1995. This cohort study followed a group of elders assessing cognition, general health, medical history, diet, pharmaceutical use, physical activity, education, etc. Of the 5,657 potential participants identified 89.7% were enrolled (n = 5092). Baseline and subsequent screening interviews included self or proxy reports of demographic variables, medical history, occupational history, smoking or alcohol history, and information on family history.

Methods and procedures were reviewed by Institutional Review Boards at Utah State, Duke, and Johns Hopkins and all participants or legal caregivers signed an informed consent to participant.

Outcome assessment

The main outcome was yes/no incident dementia and Alzheimer's disease across nine years of follow-up. Cognitive function was screened at baseline and three subsequent follow-up assessments (wave 1: 1997-1998, wave 2: 1998-2000, and wave 3: 2002-2004) using a modified version of the Mini Mental State Examination (3MS) or among those unable to complete the 3MS were rated by knowledgeable informants using an Informant Questionnaire for Cognitive Decline (IQCODE). Participants who scored lower than 87 on the 3MS or higher than 3.27 on the IQCODE and all subjects older than 90 years of age were matched with controls from the remaining cohort and were further

assessed for cognitive status using a multi-staged clinical assessments. The clinical data included results from the Dementia Questionnaire (DQ) and a clinical assessment which included an examination, blood-pressure measurement, neurologic examination, psychometric testing, and review of cognitive symptoms and medical histories. The DQ is an inventory of cognitive symptoms, functional impairments, and medical conditions relevant to dementia administered by trained nurses. The clinical evaluations were review by a geropsychiatrist and a neuropsychologist, who assigned dementia diagnoses, noncases, and other dementia diagnoses. Those assigned a dementia diagnoses were further examined by a board-certified geropsychiatrist and completed a magnetic resonance imaging scan and laboratory tests. Agreement of final diagnoses was performed by consensus conference of experts.

Diet assessment

An estimate of usual dietary intake at baseline was collected using a self-administered Food Frequency Questionnaire (FFQ). The FFQ used was modeled after the Harvard Nurses' Health Study FFQ; its design has been test and validated for replicated use (35, 36). It included sections surveying dietary supplement usage. FFQs are useful for large population-based studies due to their ability to estimate average intake across long periods of time, to be self administered, and are relatively quick and inexpensive. FFQ provide an assessment of usual dietary intake in a single administration.

Using standard FFQ methodology, participants were asked to report the frequency they consumed one of the 142 foods or food groups. The frequency of consumption

categories were as follows: Less than 1 per month, 1-3 times per month, 2-4 times per month, 1 time per week, 2-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.

To calculate intake of a specific nutrient, the nutrient content of each food is multiplied by the frequency of consumption for each food. Nutrient intake is then is summed over all food items for each individual. A database containing foods reported by participants in the CCMS with nutrients per 100 grams was created. Nutrient composition information was obtained from standard nutrient databases like the Food Processor (ESHA version 8.2) and time matched to the dates of the study (37). The ESHA Food Processor version 8.2 (used for wave 1) consists of approximately 30,000 foods (there are >32,000 foods in more current versions) and is updated frequently. It includes specific brands and types of foods such as "Wendy's chili" or "Kellogg's Corn Flakes," many of these foods are not available through the USDA. Approximately ¼ of the foods come from the USDA nutrient composition database, the rest come from manufacturers and restaurants (37).

The openware program known as FoodCalc, was used for quantification of the FFQ data. The computational tool is public-domain software from the Danish Cancer Registry written by Jesper Lauritsen, and is an effective tool for evaluating large amounts of FFQ data (38). Once a table of foods representative of the foods in the FFQ is compiled, Foodcalc will total nutrients by individuals intake per day, by specific foods or food groups, or overall totals by all individuals for all foods consumed (38).

Nutrient intake was adjusted for total energy intake using the residual method outline by Walter Willet (39). Kilocalories are used as the independent variable and

absolute nutrient intake from food as the dependent variable in a regression model. Energy adjusted intakes of the nutrient of interest are calculated using residuals from the model summed with the mean caloric intake level of that nutrient (39). Energy adjusted levels were summed with nutrient intake from supplements to obtain total intake of nutrients.

At baseline 5092 participants were screened using the 3MS; those who scored below the cutoff did not receive a FFQ. Of the 4737, who were asked to complete the FFQ, 3829 (81%) completed and returned the questionnaire. Of those, 197 were excluded based on later clinical diagnosis of prevalent dementia or implausible caloric intake (energy intake greater than 5000 kilocalories or less than 500 kilocalories per day). Thus, 3634 participants who were not demented and provided complete dietary data at the baseline interview are included in the analyses presented here. In subsequent analyses an additional 116 participants with incidence of non-AD type dementia were also excluded, 3518 participants remained for the incident AD analysis.

Statistical analysis

The SPSS version 15.0 for Windows software program was used for all statistical analysis. Exposure variables were defined using quintiles of total (food plus supplement) folate, B-12, and B-6 and folate and B-6 from food sources only. Quintiles of B-vitamin intake were used because usual dietary intake was assessed using an FFQ which has better power to rank levels of intake of individuals than to assess actual intake levels. Quintiles were assigned using a visual binning tool in the SPSS software program. Additionally, degree of supplementation with folic acid and vitamin B-6 was

defined as more or less than 400 µg of folic acid/day or more or less than 2 mg of vitamin B-6/day. Descriptive analyses were conducted using chi-square tests of independence for categorical variables and one-way ANOVAs to examine continuous variables. Cox proportional Hazards modeling was used to evaluate risk of incident dementia and AD across increasing quintiles of total folate intake and other B-vitamin intake. The time variable was defined as the age at event defined by the participant's age at diagnoses of dementia, their current age if non-demented and remaining in the study and for those censored and no longer in the study, their age at death or last follow-up. The event variable was incident dementia or AD (dementia/AD=1 and noncase=0).

Covariates used in the analysis were obtained from the baseline interview and included gender, level of education (less than high school education or greater than high school education), APOE genotype defined by (no copies or 1-2 copies), history of tobacco (ever/never) and alcohol use (ever/never), physical activity pattern (more than a few times per month or more or less than a few times per month), total caloric intake (kcal/day), self-reported comorbidities (history of diabetes (ever/never), myocardial infarction (ever/never), and stroke (no/probable) at baseline), and related dietary factors (folate, B-12, or B-6). Associated dietary factors were included as continuous variables in the model of the main effect of other dietary factors.

The Likelihood Ratio Test (LRT) was used to examine the fit of the models examining incident AD. In the fully adjusted model (model 3), including total folate did not improve the fit of the model (p=0.67). The same was true in models with folate from food only and in the model with folate from supplements only (p=0.30 and p=0.52 respectively). LRT also revealed no improvement in the strength of model 2 using total

folate, food folate, or folate from supplements (p=0.76, p=0.31, and p=0.59, respectively). Including total B-12 and B-6 in models two and three did not improve fit of the models either.

RESULTS

Two-hundred and two of the participants remaining in our analysis had developed Alzheimer's disease by wave three. **Table 3.1** illustrates characteristics of the population by gender. A larger proportion of women than men were enrolled in the study. Women in the CCMS had higher rates of supplement use, 46% versus 39% ($P < 0.001$) and a significantly larger proportion had completed more than a high school education at 87% versus 82% ($P < 0.001$). They also had significantly higher rates of incident AD at 3.5% versus 2.0% ($P = 0.042$). Men in the CCMS were significantly younger ($P < 0.001$), consume more kilocalories ($P < 0.001$), had higher rates of MI at 17.5% versus 8.8% ($P < 0.001$) and also had higher rates of tobacco ($P < 0.001$) and alcohol usage ($P < 0.001$). Men also exercised more often than women at 89% versus 83% ($P < 0.001$).

Folic acid supplement use was defined as daily consumption of 400 µg of supplemental folic acid or more. Folic acid supplement users were more likely to be women, have higher baseline 3MS scores, weigh less, and have better dietary patterns including eating less fat, more fiber, fruit and vegetables, and fish. Folic acid supplement users were also more likely to have at least a high school education and to exercise more than were non supplement users (**Table 3.2**). A high percentage of participants in Q4-5 of total folate (food plus supplement) consumed supplements of folic acid and thus those in Q4-5 of total folate were more likely to be women, had more education, and eat

healthy diets with fruits, vegetables, and fish. **Table 3.3** illustrates population characteristics by quintiles of folate intake from food and supplemental sources. Those in the top quintiles of folate intake also consumed more fruits, vegetables, and fish.

TABLE 3.1
Population characteristics by gender

	Male (n=1564)	**Female** (n=2070)
Food folate intake (µg/d)[2]	319 ± 126	321 ± 134
Food B-12 intake (µg/d)[2]	5.99 ± 3.76	6.27 ± 4.69
Food B-6 intake (mg/d)[2]	2.11 ± 0.70	2.27 ± 0.70***
Total folate plus supplement intake (µg/d)[2]	477 ± 256	506 ± 256***
Total B-12 plus supplement intake (µg/d)[2]	9.18 ± 10.96	10.00 ± 9.52*
Total B-6 plus supplement intake (mg/d)[2]	6.65 ± 18.27	7.87 ± 21.38
Dementia (%)	3.5	5.3
Alzheimer's (%)	2.0	3.5*
BMI (kg/m²)	26.40 ± 3.87	26.05 ± 4.82*
Age (baseline)	74.19 ± 6.53	75.00 ± 6.75***
Total energy intake (kcals/d)	2049 ± 786	1882 ± 764***
Multivitamin supplement users (%)	38.6	45.8***
Folic acid supplement users (%)	4.5	4.3
B-Complex vitamin supplement users (%)	9.0	12.1**
Vitamin B-6 supplement users (%)	7.9	8.4
Use Tobacco (% ever)	34.8	6.9***
Use Alcohol (% ever)	27.3	7.4***
Moderate Physical Activity (% Yes[3])	89.0	83.0***
Stroke (% Probable/Uncertain)	4.5	3.5
MI (% at baseline)	17.5	8.8***
Diabetes (% at baseline)	14.0	12.0
ApoE alleles (% 1-2 copies)	32.3	30.9
Education (% >High School)	81.5	86.6***

[1] $\bar{x}$ ± SD (all such values).
[2] Energy adjusted nutrients from food
[3] Yes = a few times a month or more
*p<0.05
**<0.01
***p<0.001

TABLE 3.2
Population characteristics by folic acid supplement intake from any supplement source

	No (< 400 µg/d) (n=2207)	**Yes** (>/=400 µg/d) (n=1427)
Male (%)	45.7	39.0*
Dementia (%)	8.7	8.9
Alzheimer's (%)	5.5	5.9
Average 3MS		
Wave 1	90.97 ± 6.70	91.52 ± 6.14**
Wave 2	91.72 ± 7.96	92.34 ± 7.70*
Wave 3	90.18 ± 9.24	90.68 ± 8.28
BMI (kg/m²)	26.38 ± 4.52	25.94 ± 4.29**
Age (baseline)	74.69 ± 6.80	74.60 ± 6.47
Total energy intake (kcals/d)	1936.71 ± 773.34	1980.16 ± 783.59
Total folate intake (µg/d)[1]	320.03 ± 136.16	320.91 ± 121.43
Total vitamin B-12 intake (µg/d)[1]	6.21 ± 4.20	6.05 ± 4.49
Total vitamin B-6 intake (mg/d)[1]	2.16 ± 0.65	2.26 ± 0.79***
Total CHO (g/d)[1]	264 ± 37.7	268 ± 43.9***
Total Protein (g/d)[1]	86. 5 ± 17.7	87.8 ± 19.1*
Total Fat (g/d)[1]	70.8 ± 13.3	68.4 ± 15.3***
Total Fiber (g/d)[1]	19.1 ± 6.5	20.7 ± 7.7***
Folic acid from supp (µg/d)[1]	6.5 ± 34.6	431 ± 107***
Use Tobacco (% Ever)	18.5	19.6
Use Alcohol (% Ever)	84.3	83.6
Moderate Physical Activity (% Yes[3])	84.2	87.7**
Stroke (% Probable/Uncertain)	4.7	2.8*
MI (% at baseline)	13.6	11.0*
Diabetes (% at baseline)	13.3	12.1
ApoE alelles (% 1-2 copies)	31.7	31.0
Education (% > High School)	83.0	86.6**
Servings of Fruit/day	2.53 ± 1.71	2.91 ± 2.24***
Servings of Vegetables (no potato)/day	3.49 ± 2.49	3.89 ± 2.76***
Servings of Dairy/day	2.20 ± 1.51	2.25 ± 1.52
Servings of Fish/day	0.20 ± 0.22	0.22 ± 0.22**
Servings of Grain/day	3.10 ± 1.70	3.05 ± 1.65
Servings of Sweets/day	0.95 ± 1.06	0.90 ± 1.00
Servings of Meat and Poultry/day	0.98 ± 0.65	1.02 ± 0.74

[1]Energy adjusted nutrients from food and supplement (when applicable)
[2]$\bar{x}$ ± SD (all such values).
[3]Yes = a few times a month or more
*p<0.05 for difference across quintiles
**<0.01 for difference across quintiles
***p<0.001 for difference across quintiles

TABLE 3.3
Population characteristics by quintiles of folate intake from food and supplemental sources[1]

	Quintiles				
	1 (n=727)	**2** (n=727)	**3** (n=727)	**4** (n=727)	**5** (n=726)
Total folate (µg/d)[1]	212 ± 56	303 ± 22	427 ± 66	656 ± 39	870 ± 178***
Male (%)	45.8	47.5	43.9	39.8	38.3**
Dementia (%)	9.5	8.3	8.4	7.3	10.3
Alzheimer's (%)	5.9	6.1	6.3	4.5	5.9
Average 3MS					
Wave 1	90.6 ± 6.7	91.3 ± 6.5	91.2 ± 6.5	91.6 ± 6.5	91.3 ± 6.2
Wave 2	92.3 ± 8.4	92.3 ± 7.7	91.8 ± 7.6	92.6 ± 8.0	92.2 ±7.6
Wave 3	89.5 ± 10.3	90.6 ± 8.6	90.1 ± 9.0	91.6 ± 7.4	90.5 ± 8.7
BMI (kg/m²)	26.5 ± 4.7	26.3 ± 4.5	26.4 ± 4.4	26.1 ± 4.2	25.8 ± 4.4
Age (baseline)	74.6 ± 6.9	74.6 ± 6.7	74.7 ± 6.7	74.3 ± 6.4	75.0 ± 6.6
Total energy (kcals/d)	1931 ± 752	1956 ± 787	1909 ± 766	1971 ± 818	2001 ± 762
MVM supp users (%)	1.7	2.4	21.5	90.3	94.6***
Folic acid supp users (%)	0.0	0.0	0.1	2.9	19.1***
B-Complex supp users (%)	4.8	4.7	6.6	13.9	23.8***
Vit B-6 supp users (%)	3.0	2.6	3.8	9.8	21.2***
Use Tobacco (% Ever)	20.3	19.4	15.5	18.9	20.4
Use Alcohol (% Ever)	15.9	16.0	14.7	14.0	17.2
Mod Physical Activity (% Yes[3])	85.3	83.8	85.3	87.3	86.2
Stroke (% Probable/Uncertain)	4.0	4.0	5.4	2.6	3.5
MI (% at baseline)	14.1	13.0	13.3	12.5	10.0
Diabetes (% baseline)	13.2	14.2	12.1	11.9	12.8
ApoE alleles (1-2 copies)	35.3	31.2	28.7	30.7	31.4*
Education (% > High School)	80.3	84.8	85.1	86.9	84.8*
Servings of Fruit/day	2.55 ± 1.75	2.53 ± 1.67	2.55 ± 1.74	2.77 ± 2.51	3.00 ± 1.89***
Servings of Vegetables (no potato)/day	3.53 ± 2.67	3.53 ± 2.50	3.41 ± 2.35	3.81 ± 2.66	3.95 ± 2.79***
Servings of Dairy/day	2.18 ± 1.49	2.26 ± 1.52	2.16 ± 1.49	2.22 ± 1.56	2.27 ± 1.50
Servings of Fish/day	0.21 ± 0.24	0.20 ± 0.20	0.20 ± 0.21	0.23 ± 0.21	0.23 ± 0.23*
Servings of Grain/day	3.12 ± 1.68	3.10 ± 1.70	3.06 ± 1.74	3.06 ± 1.71	3.06 ± 1.58
Servings of Sweets/day	0.95 ± 1.11	0.94 ± 1.01	0.92 ± 0.93	0.94 ± 1.09	.90 ± 1.02
Servings of Meat & Poultry/day	0.95 ± 0.66	1.01 ± 0.65	0.97 ± 0.63	1.02 ± 0.71	1.02 ± 0.77

[1]Energy adjusted nutrients from food and supplement
[2]$\bar{x}$ ± SD (all such values).
[3]Yes = a few times a month or more
*p<0.05 for difference across quintiles
**<0.01 for difference across quintiles
***p<0.001 for difference across quintiles

Table 3.4 illustrates nutrients intakes and food group patterns across quintiles of folate intake from food only. Interestingly, there are no significantly differences between intake of nutrients or foods across quintile of intake of folate from food. Top contributors of folate intake included ready-to-eat-cereal (RTEC), orange juice, bananas, romaine lettuce, and wheat bread. There was no difference in risk across increasing quintiles of folate intake.

Cox proportional Hazards models were used to examine associations between B-vitamins and incident dementia/AD. No associations were observed between increasing quintiles of total folate and incident dementia or with AD in either unadjusted or adjusted models (**Table 3.5-3.7**). Fully adjusted models included gender, education, number of apoE alleles, bmi, total calories, physical activity, history of alcohol and tobacco use, history of myocardial infarction, diabetes, stroke, and other B-vitamins (B-12, B-6). In similar analyses total B-12 and B-6 from food and supplements combined were also not associated with incident dementia and AD adjusted or unadjusted models.

These analyses were repeated using quintiles of folate and B-6 from food only. No associations existed between both dementia and AD. Similarly, no associations were observed in models with supplemental folate and B-6.

TABLE 3.4
Nutrient intake by quintiles of folate intake from food[1]

	Quintiles				
	1 (n=727)	**2** (n=727)	**3** (n=727)	**4** (n=727)	**5** (n=726)
Total Folate intake (µg/d)[1]	185 ± 56	258 ± 13	302 ± 13	354 ± 19	506 ± 157***
Total Energy intake (kcal/d)	1910 ± 764	1963 ± 820	1967 ± 820	1963 ± 778	1966 ± 779
Total Fat intake (g/d)[1]	69.9 ± 14.1	69.7 ± 14.0	69.7 ± 14.7	69.8 ± 14.2	70.4 ± 13.8
Total Protein intake (g/d)[1]	87.3 ± 17.3	86.9 ± 18.5	87.0 ± 17.8	87.5 ± 18.8	86.2 ± 19
Total CHO intake (g/d)[1]	265 ± 38.5	266 ± 41.2	265 ± 44.2	264 ± 39.0	266 ± 38
Total Fiber intake (g/d)[1]	19.6 ± 7.0	19.9 ± 7.3	19.8 ± 7.8	19.8 ± 6.8	19.8 ± 6.8
Total Vit C intake (mg/d)[1]	328 ± 328	338 ± 356	342 ± 361	342 ± 335	335 ± 345
Total Iron Intake (mg/d)[1]	25.1 ± 34.3	24.6 ± 34.2	25.0 ± 38.0	27.6 ± 41.8	24.9 ± 34.6
Total Vit B-12 (µg/d)[1]	6.2 ± 4.2	5.9 ± 3.3	6.3 ± 4.5	6.3 ± 4.4	6.2 ± 5.0
Total Vit B-6 (mg/d)[1]	2.2 ± 0.7	2.2 ± 0.7	2.2 ± 0.7	2.3 ± 0.9	2.2 ± 0.6
Servings of Fruit/day	2.9 ± 1.4	3.0 ± 1.4	3.0 ± 1.4	3.1 ± 1.4	3.0 ± 1.4
Servings of Vegetables (no potato)/day	3.0 ± 1.5	3.1 ± 1.4	3.0 ± 1.4	3.0 ± 1.4	3.0 ± 1.4
Servings of Dairy/day	2.2 ± 1.5	2.3 ± 1.5	2.2 ± 1.6	2.3 ± 1.6	2.1 ± 1.4
Servings of Fish/day	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Servings of Grain/day	3.1 ± 1.7	3.1 ± 1.7	3.1 ± 1.7	3.1 ± 1.7	3.1 ± 1.7
Servings of Sweets/day	0.9 ± 1.1	0.9 ± 1.0	0.9 ± 1.0	0.9 ± 0.9	1.0 ± 1.2
Servings of Meat & Poultry/day	1.0 ± 0.7	1.0 ± 0.6	1.0 ± 0.7	1.0 ± 0.8	1.0 ± 0.6
MVM supp users (%)	40.8	42.5	43.1	44.5	42.7
Folic acid supp users (%)	3.3	4.6	4.3	4.9	4.8
B-Complex supp users (%)	10.3	10.3	10.1	11.7	11.2
Vitamin B-6 supp users (%)	8.6	8.5	7.4	8.2	8.0

[1]Energy adjusted nutrients from food sources only
[2]$\bar{x}$ ± SD (all such values).
*p<0.05 for difference across quintiles
**<0.01 for difference across quintiles
***p<0.001 for difference across quintiles

TABLE 3.5
Hazard ratios (95% CIs) for quintiles of total folate, vitamin B-12, and vitamin B-6 intake over 9 years of dementia or AD incidence

Quintiles of Total Folate Intake

	1	2	3	4	5	p-trend
Dementia						
Model 1	Ref	0.91 (0.64, 1.29)	0.88 (0.63, 1.25)	0.83 (0.58, 1.19)	1.07 (0.74, 1.54)	0.85
Model 2	Ref	0.89 (0.63, 1.25)	0.86 (0.61, 1.22)	0.80 (0.56, 1.15)	1.04 (0.75, 1.45)	0.89
Model 3	Ref	0.88 (0.62, 1.28)	0.89 (0.61, 1.28)	0.76 (0.51, 1.12)	1.08 (0.76, 1.55)	0.81
AD						
Model 1	Ref	1.08 (0.71, 1.66)	1.06 (0.69, 1.63)	0.84 (0.53, 1.33)	0.99 (0.64, 1.52)	0.60
Model 2	Ref	1.04 (0.68, 1.60)	1.02 (0.67, 1.56)	0.79 (0.50, 1.25)	0.93 (0.60, 1.44)	0.45
Model 3	Ref	1.04 (0.66, 1.63)	1.03 (0.66, 1.62)	0.75 (0.45, 1.23)	0.97 (0.61, 1.54)	0.53

Quintiles of Total B-12 Intake

	1	2	3	4	5	p-trend
Dementia						
Model 1	Ref	1.18 (0.82, 1.69)	1.44 (1.03, 2.01)	1.04 (0.67, 1.40)	1.13 (0.80, 1.62)	0.85
Model 2	Ref	1.18 (0.82, 1.69)	1.42 (1.02, 1.99)	0.95 (0.65. 1.37)	1.13 (0.79, 1.61)	0.92
Model 3	Ref	1.16 (0.79, 1.70)	1.33 (0.93, 1.92)	0.93 (0.60, 1.42)	1.03 (0.68, 1.56)	0.87
AD						
Model 1	Ref	1.06 (0.67, 1.68)	1.51 (1.00, 2.29)	0.96 (0.60, 1.52)	1.12 (.073, 1.73)	0.80
Model 2	Ref	1.05 (0.66, 1.67)	1.48 (0.98, 2.24)	0.92 (0.58, 1.46)	1.08 (0.69, 1.68)	0.96
Model 3	Ref	1.12 (0.69, 1.83)	1.47 (0.94, 2.31)	1.00 (0.58, 1.70)	1.03 (0.62, 1.73)	0.97

Quintiles of Total B-6 Intake

	1	2	3	4	5	p-trend
Dementia						
Model 1	Ref	0.94 (0.66, 1.34)	1.01 (0.72, 1.42)	0.99 (0.69, 1.40)	0.92 (0.64, 1.30)	0.75
Model 2	Ref	1.10 (1.57, 0.77)	1.03 (1.48, 0.72)	1.10 (1.55, 0.78)	1.06 (1.51, 0.75)	0.69
Model 3	Ref	1.03 (0.71, 1.51)	0.96 (0.66, 1.40)	0.94 (0.61, 1.45)	0.93 (0.59, 1.46)	0.98
AD						
Model 1	Ref	1.23 (0.53, 1.26)	1.20 (0.55, 1.28)	1.08 (0.60, 1.41)	1.22 (0.53, 1.27)	0.58
Model 2	Ref	1.27 (0.82, 1.97)	1.02 (0.65, 1.60)	1.03 (0.66, 1.60)	1.10 (0.71, 1.71)	0.42
Model 3	Ref	0.85 (0.54, 1.35)	0.74 (0.46, 1.19)	0.92 (0.55, 1.56)	0.82 (0.47, 1.43)	0.49

Model 1- unadjusted
Model 2- adjusted for gender, education
Model 3- fully adjusted for gender, education, bmi (cont), total kcals (cont), physical activity, apoe, alcohol, smoking, MI, stroke, DM, and other B-vitamins scored in quintiles and treated as a continuous variables (18 = df).

TABLE 3.6
Hazard ratios (95% CIs) for quintiles of food folate and vitamin B-6 intake over 9 years of dementia or AD incidence

Quintiles of Food Folate Intake

	1	2	3	4	5	p-trend
Dementia						
Model 1	Ref	0.95 (0.65, 1.40)	1.08 (0.75, 1.57)	1.23 (0.86, 1.76)	0.86 (0.58, 1.26)	0.93
Model 2	Ref	1.00 (0.70, 1.43)	1.00 (0.71, 1.43)	1.22 (0.87, 1.70)	0.86 (0.60, 1.24)	0.84
Model 3	Ref	0.99 (0.67, 1.45)	1.05 (0.72, 1.52)	1.27 (0.89, 1.82)	0.88 (0.60, 1.29)	0.97
AD						
Model 1	Ref	1.17 (0.73, 1.87)	1.12 (0.70, 1.79)	1.45 (0.93, 2.26)	1.54 (1.00, 2.38)	0.94
Model 2	Ref	0.96 (0.06, 1.53)	1.20 (0.78, 1.85)	1.28 (0.83, 1.97)	0.84 (0.52, 1.34)	0.95
Model 3	Ref	0.96 (0.58, 1.60)	1.27 (0.80, 2.01)	1.35 (0.86, 2.14)	0.91 (0.56, 1.50)	0.77

Quintiles of Food B-6 Intake

	1	2	3	4	5	p-trend
Dementia						
Model 1	Ref	0.91 (0.63, 1.33)	1.04 (0.73, 1.48)	0.78 (0.53, 1.14)	0.92 (0.65, 1.31)	0.53
Model 2	Ref	1.12 (0.80, 1.57)	1.00 (0.70, 1.43)	1.13 (0.81, 1.59)	0.94 (0.66, 1.34)	0.46
Model 3	Ref	1.23 (0.85, 1.78)	1.10 (0.75, 1.61)	1.37 (0.96, 1.97)	1.01 (0.70, 1.48)	0.22
AD						
Model 1	Ref	0.95 (0.60, 1.52)	1.24 (0.80, 1.92)	1.32 (0.86, 2.02)	0.85 (0.53, 1.37)	0.97
Model 2	Ref	0.76 (0.48, 1.21)	1.05 (0.70, 1.59)	0.69 (0.44, 1.09)	0.96 (0.64, 1.44)	0.75
Model 3	Ref	0.81 (0.49, 1.34)	1.21 (0.77, 1.90)	0.72 (0.44, 1.18)	0.89 (0.56, 1.40)	0.48

Model 1- unadjusted
Model 2- adjusted for gender, education
Model 3- fully adjusted for gender, education, bmi (cont), total kcals (cont), physical activity, apoe, alcohol, smoking, MI, stroke, DM, and other B-vitamins scored in quintiles and treated as a continuous variables (18 = df).

TABLE 3.7
Hazard ratios (95% CIs) for supplemental folate and vitamin B-6 intake over 9 years of dementia or AD incidence

	Supplemental Folate Intake*		
	1	**2**	**p-trend**
Dementia			
Model 1	Ref	1.08 (0.85, 1.37)	0.69
Model 2	Ref	1.03(0.82, 1.29)	0.78
Model 3	Ref	1.04 (0.81, 1.33)	0.76
AD			
Model 1	Ref	0.96 (0.72, 1.27)	0.74
Model 2	Ref	0.93 (0.69, 1.23)	0.39
Model 3	Ref	0.90 (0.66, 1.23)	0.52
	Supplemental B-6 Intake**		
	1	**2**	**p-trend**
Dementia			
Model 1	Ref	1.00 (0.79, 1.26)	0.77
Model 2	Ref	1.03 (0.82, 1.29)	0.73
Model 3	Ref	1.06 (0.77, 1.47)	0.72
AD			
Model 1	Ref	0.92 (0.70, 1.22)	0.83
Model 2	Ref	0.96 (0.60, 1.53)	0.79
Model 3	Ref	0.99 (0.66, 1.50)	0.96

*Defined by supplemental intake of folic acid 1= <400µg/d, 2= >/= 400µg/d
**Defined by supplemental intake of B-6 1= <2mg, 2 >2mg
Model 1- unadjusted
Model 2- adjusted for gender, education
Model 3- fully adjusted for gender, education, bmi (cont), total kcals (cont), physical activity, apoe, alcohol, smoking, MI, stroke, DM, and other B-vitamins scored in quintiles and treated as a continuous variables (14 = df).

DISCUSSION

Usual dietary intake of folate, vitamin B-12, and vitamin B-6 was not associated with risk for incident dementia or AD over 9 years among CCMS elders. In models with the b-vitamins together, no association was found. Neither folic acid nor B-6 from food or supplement intake was related to incident dementia and AD. Vitamin B-12 from supplements was not analyzed, as oral B-12 supplementation is often given in high doses

(~1000 µg) in response to B-12 deficiency and actual absorbency varies in response to the degree of malabsorption.

Plausible biological mechanisms link folate and other B-vitamins to AD, and originate from their roles in methylation reactions of one-carbon metabolism. Deficiency of B-vitamins has been known to raise levels of homocysteine levels via disrupted one-carbon metabolism (25). It has been proposed that elevated levels of homocysteine may induce oxidative stress and increase damage to neurotoxicity (40-42). Damaged or altered environment in the brain may contribute to increased plaques and tangles observed in persons with AD. In addition to elevated homocysteine levels, other consequences of disturb one-carbon metabolism include decrease levels of S-adenosyl-methionine (SAM), increased S-adenosylhomocysteine (SAH), limitation of certain folate metabolites (25, 43).

SAM is required for methylation reactions of DNA/RNA and neurotransmitters, specifically it functions affect proteins, membrane phospholipids, neurotransmitters, and DNA structure. In adequate levels of SAM may alter normal brain functions or damage vital structures for normal cognitive function (25, 43). SAH the precursor to homocysteine, has also been observed at irregular levels in individuals with AD similar to homocysteine, SAM and B-vitamins like folate, B-12, and B-6 (44). It is thought to increase as homocysteine metabolism is inhibited. SAH is an intermediate between SAM and homocysteine. One study noted that increases in SAH inhibited normal functions of SAM in the brain (6).

Vitamin B-12 is a cofactor for methionine synthase, the enzyme that transfers a methyl group from 5-methyl-THF to homocysteine to form methionine. The conversion

of 5,10 methylene-THF to 5-methyl-THF requires the enzyme, methyltetrahydrofolate reductase. This reaction is irreversible, therefore deficiency of B-12, prevents 5-methyl-THF from being converted forward to other folate forms or back to 5,10 methylene-THF. Lack of these other essential cofactor forms of folate may cause breaks in DNA, which may influence damage common in AD patients brains (43).

Homocysteine can be metabolized via two different pathways, the previously mentioned reaction requiring 5-methyl-THF and B-12 and an althernative pathway which requires B-6 as a cofactor in converting homocysteine to cystathioine. Further, downstream of this pathway glutathione is produced, a key antioxidant for preventing lipid peroxidation and other oxidative damage. Therefore, disrupted brain function may also be related to the decreased production of glutathione via metabolism of homocysteine to cystathione.

Prevalence of B-vitamin deficiencies, especially B-12, are higher among elderly populations. Conditions which inhibit vitamin B-12 absorption such as gastritis have been estimated to affect 20-50% of the elderly in the US. Among the Framingham group 24% of elders age 60-69 and 37% of those older than 80 were found to have gastritis depending upon diagnosis criteria followed (6). Deficiency of folate is also suspected to be higher among elderly groups and may increase with age due to decreased absorption caused by changes in the gastrointestinal tract even studies examining folate intake post-fortification have also seen average intakes below recommendations (26, 45).

In 1998, a mandate requiring all cereal grains to be fortified with folic acid was instated in response to research on folate status and neural tube defects in pregnant women. Standardized levels of 140 µg per 100 grams of grain was required (46). Some

have raised concern that the benefits may not reach all populations and may even be detrimental to subgroups (33, 47). Mechanistically, concern is derived from the concept that high levels of folic acid may "mask" evidence of vitamin B-12 deficiency (30, 31). Macrocytic anemia is a common consequence of deficiency of either B-12 or folate. Clinically, supplementation with folic acid is often the treatment prescribed for macrocytic anemia, such treatment strategies may alleviates symptoms of anemia while further camouflaging B-12 deficiencies. Prolonged B-12 deficiency has been seen to cause cognitive deficits and in certain cases, depending upon severity and duration of the deficiency, has been irreversible (26).

Since the time of mandatory folate fortification, increases in serum levels of folate have been observed indicating that though many products were already fortified with folic acid, the mandate did meet its goal of increasing average intakes of folic acid. The Framingham group observed a 38% increase of serum RBC folate among participants who did not consume supplements post-fortification; they also estimated a 3% less of there cohort were deficient in folate post-fortification compared to pre-fortification (30). As the top contributor to folate, RTEC has been studied since the fortification of cereal grains with folic acid. Findings which suggest vitamins fortified in foods like RTEC may be more bioavailable than naturally occurring sources of folate (48). If folate was independently associated to increased risk for cognitive decline and AD, a decrease or slow in incidence would have been expected. No such report or observation has been made. One study, by Morris et al. (47) did report increased risk for anemia and cognitive decline among those with low serum B-12 and high serum folate. The study included 1459 senior participants in the 1999-2002 US National Health and Nutrition Examination

Survey. Morris et al. (33) also found high folate intake to be associated with faster cognitive decline among a group of elderly from the Chicago Health and Aging Project. These finding support concerns that high folate may be toxic in certain situations.

It appears that dietary folate is not a marker of a dietary pattern and is somewhat ubiquitously distributed in a wide variety of foods since the fortification of the US food supply with folic acid. In 1995, the CCMS participants averaged intake of folate from food was below the RDA (400 µg/d) at 319 µg/d and 46.9 % received less than the RDA including folate from supplements and 77.1% received less than the RDA from food folate. This data was collected pre-folate fortification and higher intakes could be expected in most participants post-fortification. It should also be noted that dietary intake was collected using FFQ's; FFQ's are better at detecting relative intake comparing individuals and lack the ability to assess actual intake. An analysis of participants with low serum levels and inadequate dietary intake of B-vitamins may offer more insight to mediating effects of B-vitamins at different levels of intake. Associations between dietary intake and AD may be difficult to detect as dietary intake in elderly may not indicate actual nutriture. Increases in malabsorption related to gastritis or pernicious anemia contribute to the problem of using dietary data alone to assess possible associations between B-vitamins and AD.

It is not surprising that results from population-based prospective studies on B-vitamins and cognitive health vary significantly. The studies range in examining combinations of B-vitamins from food, supplements, total, related biomarkers and outcome variables of cognitive decline, incident dementia, and incident AD. Additionally methods of analyzing dietary intake, nutriture, cognition, and type of

dementia have been inconsistent. Results from this study appear consistent with some (34) but not all recently published data (5, 49) similar in design. There is little information on dietary intake of B-vitamins and AD (5, 34, 49). Corrado et al. (50) published an analysis that found no independent relation between total B-12 with incident of AD in the Baltimore Longitudinal Study of Aging (BLSA). The BLSA is a population based cohort study of elderly men and women from the Baltimore area. Higher folate was associated with decreased risk for incident AD (RR: 0.45; 95% CI: 0.2, 0.97) in an adjusted model with age, gender, education, and caloric intake. Participants in the BLSA had similar methods to the CCMS with the use of proportional hazards modeling over an average of 9.3 years, similar to follow-up time in Cache participants. At baseline subjects in the BLSA were somewhat younger than CCMS participants (5).

Morris and colleagues conducted longitudinal assessment of incident AD across folate consumption from the Chicago Health and Aging Project (CHAP) (34). Of 1041 participants of the CHAP study, 162 developed incident AD, and similar to the results presented here, quintiles of B-vitamin intake from food and food and supplement were not associated with incidence of AD. Average intake of total folate in the CHAP was similar to the CCMS (CHAP: 338 µg/d and CCMS: 319 µg/d). Most of the CHAP dietary data was also collected pre-fortification (86%). Morris et al. had an average of 3.9 years of follow-up and used logistic regression to analyze odds between quintiles of B-vitamins and incidence of AD; the use of logistic regression does not account for censored cases in the analysis (34).

The Washington Heights Inwood Columbia Aging Project involved a smaller group (n=965) than the CCMS (49). They reported the highest quartile of folate (food

plus supplement) intake (>489.7 µg) (equivalent to quintile four in the CCMS) was related to a decrease risk for AD (HR: 0.5; 95% CI: 0.3, 0.9). Of 965 participants, 192 develop AD after 6 years; this is a much higher rate of incidence compared to the CCMS (49).

Among studies that explored serum biomarkers and incident dementia/AD results also vary. Among studies that examined homocysteine two out of three found elevated homocysteine to be related to incident AD (18, 51, 52). Wang et al. (53) examined serum folate and B-12 in relation to incident AD and found nearly double the risk for AD among subjects deficient in folate or B-12. The Conselice Study of Brain Aging (CSBA) also found low serum folate to be related to increased risk for AD (5).

The Cache Study has many strengths including a large homogenous population, lengthy follow-up time, high rates of participation and retention, a diverse and inconclusive data set of demographic information, medical history, medication usage, cognitive assessment, dietary intake, lifestyle and environment information (occupational history), etc. Limitations to this study include limitations of using a FFQ for diet assessment, a lack of associated vitamin biomarkers, and usual limitations associated with participants who drop out or are lost to follow-up. Limitations of FFQs include their ability to only detect dietary patterns and not actual intakes. Knowledge of actual nutriture via serum markers to evaluate differences in reported dietary intake and B-vitamin status would allow for better understanding of how B-vitamins may be associated with dementia and AD. FFQs are good tools for analyzing average dietary intakes over longer periods of time than 24-hour recalls or diet record, but generally are not known for detecting actual intake, as they are best designed to detect frequency. Our study's FFQ

was modeled after that of the Nurse's Health Study which has reported significant correlations between their FFQ estimates of dietary intake and estimates from dietary records collected in validation studies (35, 36). Data distinguishing between participants using B-12 supplementation or injections in response to malabsorption problems or for other purposes was not available for this analysis.

Use of the Cox Proportional Hazards statistical method is designed to help account for bias coming from censored cases like those who die prior to developing AD or those who are lost to follow-up. Logistic regression cannot account for participants who are lost to follow-up or die prior to developing dementia or AD. Several other studies examining incident AD use Cox modeling (5, 49), only one did not (34).

Several studies have looked at correlations between dietary intake and biomarkers of B-vitamin status. In the Framingham cohort an association between increasing intake of good food folate sources like breakfast cereal and fruit and vegetables was made with increased plasma folate and decreased serum homocysteine (54). Trials using un-fortified and fortified ready-to-eat breakfast cereals also saw increased serum levels of B-vitamins with increased dietary intake (55). The trial involved 215 generally healthy and disease-free elders who did not use supplements (55).

Cache populations are an interesting group to study cognitive health in because they are an unusually long lived. The 1990, US census identified Cache County as the longest lived county. Males from Cache County were estimated to live almost 10 years longer than the national average for male life expectancy. Another strength is that the participation rate was extremely high, nearly 90% at baseline and 79% during the last wave of data collection after three examinations and 9 years of follow-ups. The majority

of the population is white, members of The Church of Jesus Christ of Latter Day Saints, and consequently low rates of alcohol and tobacco use. Participants also have about an 80% rate of having at least a high school education. They are known for low rates of migration and for their tight knit families and community. The CCMS has a longer follow-up time than most studies similar in design and hypothesis. Detailed clinical assessment was used to assign diagnosis. All of these factors are benefits for longitudinal study designs.

Studies that include examining dietary B-vitamins, associated serum biomarkers, and incident dementia/AD with adequate power and follow-up time would be valuable to clarifying relationships. No studies with this design to date have been published.

CONCLUSIONS

In summary we did not find folate from food, supplements, or from combined sources to be independently associated with incident dementia or AD. Average intake of folate from food in 1995, pre-folate fortification mandate, was seen to be less than dietary recommended intakes; although average intake of folate from food and supplement combined exceeded the DRI. Further studies should be done using serum biomarkers to assess the influence and possible interactions of B-vitamin status in relation to incident dementia and AD. Some randomized trials have shown improvement of cognitive health with increased B-vitamin intake among subjects who are deficient (5, 56). Results from this study do not support the routine use of B-vitamin supplements in effort to reduce risk of dementia in general populations.

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CHAPTER 4

CONCLUSIONS

Total folate, B-12, and B-6 intake among men and women from the CCMS was not related to incidence of dementia or AD. Results from this study are not consistent with hypotheses that higher B-vitamin intake may prevent cognitive impairment and ultimately AD.

However, it is important to note that dietary intake may not adequately reflect B-vitamin status as elderly have higher rates of B-vitamin malabsorption and deficiency (1). Elderly have high rates of conditions like gastritis known to inhibit absorption of B-vitamins. In the Framingham cohort an estimated 24-37% of elderly participants may have had gastritis depending upon diagnosis criteria used (2). Conditions like pernicious anemia, use of acid blockers, and other medications also increase malabsorption of B-12 among elderly (3). Serum status may also be related to genetic variation and polymorphisms of important enzymes involved in one-carbon metabolism (4). Interactions among B-vitamins and status of other nutrients involved in methylation, not previously discussed, such as zinc, choline, and riboflavin may also affect status and normal functioning of one-carbon metabolism (5, 6).

Folate deficiency is less common since the fortification of the US food supply with folic acid (7). The average intake among CCMS participants of folate from food was below recommendations of 400 µg/d at an average of 319 µg/d pre-fortification. Of the CCMS, 46.9 % received less than the RDA including folate from supplements and 77.1% received less than the RDA from food folate alone. This may be concerning since

elderly populations have higher rates of B-vitamin malabsorption and may require higher intakes.

Studies examining B-vitamins and incidence of dementia and AD have varying results (8-10). Luchsinger et al. (9) found increased intake of folate was related to decreased risk for incident AD in a group of elderly. In the CHAP study, dietary and total B-vitamin intake was not associated with incident AD (10). And Corrado et al. (11) found folate and B-6 to be independently related to incidence of AD; B-12 was not related and in adjusted models higher folate intake was the only vitamin related to lower risk for AD.

Some studies involving subjects with prevalent B-vitamin deficiencies have seen benefits with increasing intake (8). The degree of deficiency was usually related to the amount of improvement with supplementation. Supplemental trials have also seen divided results with four of seven trials reported results (8, 12-14) and three of seven results no reported no associations (8, 14, 15). In the studies that did not see cognitive improvements decrease in homocysteine was observed, which supports some theories that homocysteine is a marker of cognitive impairment not a risk factor (8).

Folate fortification of the food supply is a concern among elderly, as it may mask a vitamin B-12 deficiency (16). Vitamin B-12 also plays a role independent of one-carbon metabolism as a cofactor in the reaction of methylmalonic acid to succinyl-CoA. Prolonged deficiency of B-12 may cause irreversible dementia, which may be related to B-12's role in methylmalonic acids conversion to succinyl-CoA (17). High intake of folate or supplementation with folic acid alleviates the most obvious symptom of B-12 deficiency, anemia, while the underlying B-12 deficiency is left untreated (16). Morris et

al. (18) observed serum levels of low B-12 and high folate to be related to increased risk for cognitive impairment. Some discussion on whether the food supply should also be fortified with B-12 has been published, most experts agree more research is needed before an informed decision can be made (19). Dietary data taken at baseline in the CCMS is pre-fortification mandate dietary data. The CCMS also collected dietary data at wave three, which occurred post-fortification (2002-2004). Comparison of these two waves of data would be an important and valuable to answer the questions surrounding folic acid, B-12 and other B-vitamin interactions.

Increased intake of B-vitamin-dense foods like fruits, vegetables, low-fat dairy and whole grains would be beneficial because of other benefits including decreased heart disease, increased weight loss/control, etc. which is associated with intake of these foods for general health, despite the lack of possible benefits in cognition. Further investigation on this topic is warranted. Large prospective studies that examine dietary, supplemental, serum biomarkers of B-vitamins and genotypes of relevant enzymes involved in one-carbon metabolism and other reactions requiring B-vitamins may concurrently be able to detect possible associations between intake/status and cognitive health.

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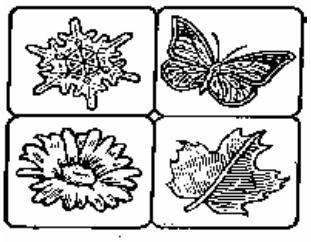
APPENDIX

APPENDIX. Wave 1 food frequency questionnaire



CACHE COUNTY STUDY ON MEMORY IN AGING
NUTRITION QUESTIONNAIRE
Conducted by: Utah State University

Marking Instructions

Please follow these few simple rules in completing this questionnaire.

1. Use only a pencil. (Please DO NOT use a pen)
2. Darken completely the circle of the answer you choose
3. Erase cleanly any answer that you wish to change
4. Make no stray marks of any kind on the form
5. For food that you never or rarely eat, please mark the first column labeled "None or
   Less than once a month. Please do not leave any food items blank.
6. Please note the correct way to mark the answers.

Correct Mark				Incorrect Mark			
❍	●	❍	❍	❍	❍	❍	❍

Please answer the following. Check the appropriate gender, and fill in your height, weight, and age

Male_____ Female_____

Height_____ Weight_____ Age_____

THANK YOU!!!!

DIETARY SUPPLEMENTS

PLEASE INDICATE WHICH, IF ANY, OF THE FOLLOWING SUPPLEMENTS YOU ARE CURRENTLY TAKING. PLEASE ANSWER "YES" OR "NO" FOR ANY SUPPLEMENT LISTED.

1. Do you regularly take multivitamins

❍ NO> PLEASE GO TO QUESTION 2

❍YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What specific brand do you use?_____

Excluding multivitamins, do you take any of the following supplements listed below?

2. Do you regularly take Vitamin A?

❍ NO> PLEASE GO TO QUESTION 3

❍ YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ less than 8,000 IU ❍ 22,001 IU or more

❍8,001 to 13,000 IU ❍ Don't know

❍ 13,001 to 22,000 IU

3. Do you regularly take Vitamin C?
❍ NO> PLEASE GO TO QUESTION 4

❍YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ less than 400 mg ❍ 1301 mg or more

❍ 401 to 700 mg ❍ Don't know

❍ 701 to 1300 mg

4. Do you regularly take Vitamin C?

❍ NO> PLEASE GO TO QUESTION 5

❍ YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ less than 100IU ❍ 504 IU or more

❍ 101 to 300 IU ❍ Don't know

❍ 301 to 500 IU

5. Do you regularly take Calcium?

❍ NO> PLEASE GO TO QUESTION 6

❍ YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ less than 400 mg ❍ 1301 mg or more

❍ 401 to 900 mg ❍ Don't know

❍ 901 to 1300 mg

6. Do you regularly take Vitamin D?

❍ NO> PLEASE GO TO QUESTION 7

❍ YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ less than 200 IU ❍ 1,000 IU or more

❍ 201 to 400 IU ❍ Don't know

❍ 401 to 1,000 IU

7. Do you regularly take Vitamin B6?

❍ NO> PLEASE GO TO QUESTION 8

❍ YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ less than 10 mg ❍ 80 mg or more

❍ 10 to 39 mg ❍ Don't know

❍ 40 to 79 mg

8. Do you regularly take Selenium?

❍ NO> PLEASE GO TO QUESTION 9

❍ YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ less than 80 mcg ❍ 251 mcg or more

❍ 81 to 130 mcg ❍ Don't know

❍ 131 to 250 mcg

FOR OFFICE USE ONLY																																	
A											B											C											
1	2	3	4	5	6	7	8	9	0		1	2	3	4	5	6	7	8	9	0		1	2	3	4	5	6	7	8	9	0	
❍	❍	❍	❍	❍	❍	❍	❍	❍	❍		❍	❍	❍	❍	❍	❍	❍	❍	❍	❍		❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
1	2	3	4	5	6	7	8	9	0		1	2	3	4	5	6	7	8	9	0		1	2	3	4	5	6	7	8	9	0	
❍	❍	❍	❍	❍	❍	❍	❍	❍	❍		❍	❍	❍	❍	❍	❍	❍	❍	❍	❍		❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	

9. Do you regularly take Iron?

❍NO> PLEASE GO TO QUESTION 10

❍YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ 50 mg or less ❍ 401 mg or more

❍ 51 to 200 mg ❍ Don't know

❍ 201 to 400 mg

10. Do you regularly take Zinc?

❍ NO> PLEASE GO TO NEXT SECTION

❍YES> CONTINUE:

(A) How many years have you taken multivitamins?

❍ 0-1 years ❍ 5-9 years

❍ 2-4 years ❍ 10 or more years

(B) What dose do you take per day?

❍ less than 25 mg ❍ 101 mg or more

❍ 26 to 75 mg ❍ Don't know

❍ 76 to 100 mg

11. DO YOU TAKE ANY OF THE FOLLOWING OTHER SUPPLEMENTS:

Cod liver oil.....❍ Yes .....❍ No	Folic acid .....❍ Yes .....❍ No
Other fish oil.....❍ Yes .....❍ No	Iodine .....❍ Yes .....❍ No
Niacin .....❍ Yes .....❍ No	Brewer's Yeast.....❍ Yes .....❍ No
Beta-caroten.....❍ Yes .....❍ No	Magnesium.....❍ Yes .....❍ No
Thiamine (vitamin B1)....❍ Yes ...❍ No	Any others?.....❍ Yes .....❍ No
B-complex vitamins .....❍ Yes ...❍ No	If yes, please specify_____

FOODS YOU EAT

For each food listed, please mark a circle for how often during the past year, on average, you have eaten the serving size specified. Be sure to mark a circle for every food listed. If you never eat the food listed mark the circle in the first column. **DAIRY FOODS**	**AVERAGE USE FOR PAST 12 MONTHS**								
	NONE OR LESS THAN 1 PER MO.	1-3 PER MO.	1 PER WK.	2-4 PER WK.	5-6 PER WK.	1 PER DAY	2-3 PER DAY	4-5 PER DAY	6 PER DAY
Skim or low fat milk (8 oz. glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Whole milk (8 oz. glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Chocolate milk or cocoa (8 oz. glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cream or half-and-half, e.g. coffee, whipped (Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Sour cream (Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Non-dairy coffee whitener (tsp)	❍	❍	❍	❍	❍	❍	❍	❍	❍

Sherbet, ice milk, or frozen yogurt (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Ice cream (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Yogurt (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cottage or ricotta cheese (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cream cheese (1 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other cheese, e.g. American, cheddar, etc., plain or as part of a dish (1 slice or 1 oz. serving)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Margarine (1 tsp, added to food or bread; exclude use in cooking	❍	❍	❍	❍	❍	❍	❍	❍	❍
Butter (1 tsp), added to food or bread; exclude use in cooking.	❍	❍	❍	❍	❍	❍	❍	❍	❍
FRUITS	❍	❍	❍	❍	❍	❍	❍	❍	❍
Raisins (1 oz. or small pack) or grapes (1/2 c)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Prunes (7 prunes or ½ cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Bananas (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍

Cantaloupe (1/4 melon)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Avocado (1/2 fruit or ½ cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Fresh apples or pears (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Apple juice or cider (small glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Oranges (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Orange Juice (small glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Grapefruit (1/2)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Grapefruit juice (small glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other fruit juices (small glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Strawberries, fresh, frozen or canned (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Blueberries, fresh, frozen or canned (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Peaches, apricots or plums (1 fresh, or ½ cup canned)	❍	❍	❍	❍	❍	❍	❍	❍	❍
VEGETABLES	❍	❍	❍	❍	❍	❍	❍	❍	❍
Tomatoes (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Tomato juice, V8 (small glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Tomato sauce (1/2 cup) e.g. spaghetti sauce	❍	❍	❍	❍	❍	❍	❍	❍	❍

Salsa or red chili sauce (1 Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Tofu or soybeans (3-4 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
String (green) beans (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Broccoli (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cabbage or cole slaw (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cauliflower (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Brussels sprouts (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Carrots, raw (1/2 carrot or 4 sticks)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Carrots, cooked (1/2 cup) or carrot juice (2-3 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Red Beets—not greens (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Corn (1 ear or ½ cup frozen or canned)	❍	❍	❍	❍	❍	❍	❍	❍	
Peas or lima beans (1/2 cup fresh, frozen, or canned)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Mixed vegetables (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Beans or lentils, baked or dried (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Dark orange (winter) squash (1/2 cup) (acorn, butternut squash)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Eggplant, zucchini or other summer squash (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Yams or sweet potatoes (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍

Spinach, cooked (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Spinach, raw as in a salad (1 cup serving)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Kale, mustard or chard greens (1/2 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Iceberg or head lettuce (1 cup serving)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Romaine or leaf lettuce (1 cup serving)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Celery (2-4 4" sticks)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Sweet green or red peppers (3 slices or ¼ pepper)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Onions as a garnish, or in salad (1 slice)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Onions as a vegetable, rings or in soup (1 onion)	❍	❍	❍	❍	❍	❍	❍	❍	❍
EGGS, MEATS, ETC.									❍
Eggs (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Chicken with skin (4-6 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Chicken without the skin (4-6 oz.), includes grilled chicken sandwich	❍	❍	❍	❍	❍	❍	❍	❍	❍
Turkey, including ground turkey (4-6 oz. or 2 turkey dogs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Hot dogs 91)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Bacon (2 slices)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Processed meats, e.g. sausage, salami, bologna,	❍	❍	❍	❍	❍	❍	❍	❍	❍

etc. (1 piece or slice)									
Hamburger (1 patty)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Taco or tostado (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Burrito (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Enchilada (2)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Beef, pork or lamb as a sandwich or mixed dish, e.g. stew, casserole, lasagna, chili etc.	❍	❍	❍	❍	❍	❍	❍	❍	❍
Pork as a main dish, e.g. ham or chops (4-6 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Beef or lamb as a main dish, e.g. steak, roast (4-6 oz)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Liver: beef, calf, or pork (4 oz)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Liver: chicken or turkey (2 oz)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Canned tuna fish (3-4 oz)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Dark meat fish, e.g. mackerel, salmon, sardines, bluefish, swordfish (3-5 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Fried fish, e.g. fish sticks, fish and chips style fish (3-5 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other fish (3-5 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Shrimp, lobster, scallops as a main dish	❍	❍	❍	❍	❍	❍	❍	❍	❍
BREADS, CEREALS, STARCHES									❍
Cold breakfast cereal (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cooked oatmeal/cooked oat bran (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍

Other cooked breakfast cereal (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Instant breakfast beverage, e.g. Carnation	❍	❍	❍	❍	❍	❍	❍	❍	❍
White bread (slice), including pita bread	❍	❍	❍	❍	❍	❍	❍	❍	❍
Dark bread (slice), including pita bread	❍	❍	❍	❍	❍	❍	❍	❍	❍
English muffins, bagels, or dinner rolls (1 each)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Muffins or biscuits (1 each)	❍	❍	❍	❍	❍	❍	❍	❍	❍
White rice (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Pasta, e.g. spaghetti, noodles, etc (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Tortillas (1-10 inch shell)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other grains, e.g. bulgur, kasha, couscous, etc (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Pancakes or waffles (2 each)	❍	❍	❍	❍	❍	❍	❍	❍	❍
French fried potatoes (4 oz. or size of small fries order)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Potatoes, baked, boiled (1each), or mashed (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Potato chips or corn chips (small bag or 1 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Crackers, e.g. Triscuits, Wheat Things (5 each)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Pizza (2 slices)	❍	❍	❍	❍	❍	❍	❍	❍	❍
BEVERAGES									❍
Plain water, bottled or tap (1	❍	❍	❍	❍	❍	❍	❍	❍	❍

cup or 8 oz. glass)									
Hawaiian Punch, lemonade, or other non-carbonated fruit drinks (1 glass, bottle, can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Low-calorie cola, e.g. Diet Coke with caffeine (can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Low-calorie caffeine-free cola (can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other low-calorie carbonated beverage, e.g. Fresca, Diet 7-UP (can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
7-Up, diet ginger ale (can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Coke, Pepsi, or other cola with sugar (can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Caffeine Free Coke, Pepsi, or other cola with sugar (can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other carbonated beverages with sugar, e.g. Sprite, Root beer (can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Regular Beer (1 glass, bottle, or can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Light Beer (1 glass, bottle, or can)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Red wine (4 oz. glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
White Wine (4 oz. glass)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Liquor, e.g. whiskey, gin, etc (1 drink or shot)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Dark tea with caffeine (1 cup), not herbal tea	❍	❍	❍	❍	❍	❍	❍	❍	❍
Green tea or herbal tea (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍

Coffee with caffeine (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Decaffeinated coffee (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
SWEETS, BAKED BGOODS, MISC									❍
Chocolate (bar or packet) e.g. Hershey's M & M's	❍	❍	❍	❍	❍	❍	❍	❍	❍
Candy bars, e.g. Snicker, milky way, Reeses	❍	❍	❍	❍	❍	❍	❍	❍	❍
Candy other than chocolate (1 oz)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cookies, home baked (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cookies, ready make (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Brownies (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Doughnuts (1)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cake, home baked (1 slice)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Cake, ready make (1 slice)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Pie, homemade (1 slice)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Pie, ready made (1 slice)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Sweet roll, coffee cake or other pastry, home baked (1 each)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Sweet roll, coffee cake or other pastry, ready made (1 each)	❍	❍	❍	❍	❍	❍	❍	❍	❍

Jams, jellies, preserves, syrup, or honey (1 Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Peanut butter (1 Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Popcorn (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Peanuts (small packet or 1 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other nuts (small packet or 1 oz.)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Oat bran, added to food (1 Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other bran, added to food (1Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Wheat germ (1 Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Chowder or cream soup (1 cup)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Olive oil salad dressing (1 Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other oil and vinegar dressing, e. g. Italian (1 Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Mayonnaise or other creamy salad dressing (1 Tbs)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Salt added at table (1 shake)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Garlic (1 clove or 4 shakes)	❍	❍	❍	❍	❍	❍	❍	❍	❍

FOOD PREPARATION	
1.	Do you eat cold breakfast cereal? ❍ NO> PLEASE GO TO NEXT QUESTION ❍ YES> What kind do you usually eat?_____

2.	How many teaspoons of sugar do you add to your beverages or food each day? ❍ 0-1 ❍ 2-4 ❍ 5-9 ❍10 or more
3.	When you have beef or lamb as a main dish, how is the meat cooked? ❍ Rare ❍ medium ❍ well ❍ Medium rare ❍ medium well ❍ do not eat meat
4.	How much of the visible fat on your beef, pork, or lamb do you remove before eating? ❍ remove all visible fat ❍ remove none ❍ remove most visible fat ❍do not eat meat ❍ remove small part of visible fat
5.	How often do you eat food that is fried at home? (exclude Pam-type spray) ❍ less than once per week ❍ 4-6 times per week ❍ 1-3 times per week ❍ daily
6.	How often do you eat fried food away from home? (e.g. french fries, fried chicken, fried fish). ❍ less than once per week ❍ 4-6 times per week ❍ 1-3 times per week ❍ daily
7.	What type and brand of cooking oil or fat do you usually use at home (e.g. corn oil, Mazola brand; lard) Type:_____ Brand:_____
8.	How does the amount of food you eat now compare to the amount you ate five years ago? ❍ I eat almost the same ❍ I eat less now

	❍ I eat more now
9.	What was the main source of your drinking water over the past year? ❍ city system ❍ rural or county system ❍ private well ❍ bottled water ❍ other (please specify_____)

FOR OFFICE USE ONLY		
(D) 1 2 3 4 5 6 7 8 9 0 ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ 1 2 3 4 5 6 7 8 9 0 ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍	(E) 1 2 3 4 5 6 7 8 9 0 ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ 1 2 3 4 5 6 7 8 9 0 ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍	(F) 1 2 3 4 5 6 7 8 9 0 ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ 1 2 3 4 5 6 7 8 9 0 ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍ ❍

YOUR ACTIVITES

1. About how many hours per day do you spend in light activity, such as walking, shopping, child care, cooking, carrying light objects, cleaning, and repairing?

   Hours per day_____

2. About how often do you take part in moderate physical activities including bowling, golf, light swimming, gardening, walks over 15 minutes, fishing, light bicycling, or other light sports.

   ❍Usually every day

   ❍ 2-6 times a week

   ❍ About once a week

   ❍ A few times a month

   ❍ A few times a year

   ❍ Rarely or never

3. About how often do you take part in vigorous physical activity including jobbing, tennis, racquetball or squash, lap swimming, aerobics, vigorous bicycling, skiing, hiking, hunting or other vigorous sports…

   ❍Usually every day

   ❍ 2-6 times a week

   ❍ About once a week

   ❍ A few times a month

   ❍ A few times a year

   ❍ Rarely or never

4. How often do you talk on the telephone with family, friends, or neighbors?

   ❍Usually every day

❍ 2-6 times a week

❍ About once a week

❍ A few times a month

❍ A few times a year

❍ Rarely or never

5. How often do you get together with family, friends, or neighbors? This includes meeting in your own home, meeting in other's homes, or going out together.

❍Usually every day

❍ 2-6 times a week

❍ About once a week

❍ A few times a month

❍ A few times a year

❍ Rarely or never

6. How often do you attend meetings of social clubs, groups, or organizations such as bridge clubs, book clubs, hospital volunteer, gardening clubs, Rotary club, Kiwanis, VFW, etc.

❍Usually every day

❍ 2-6 times a week

❍ About once a week

❍ A few times a month

❍ A few times a year

❍ Rarely or never

Thank you for completing this questionnaire. Please make sure that no questions or pages have been skipped. Please place it in the postage-paid envelope that has been provided and seal it. Please return it to us in the mail.

Thank you for your time and cooperation. You have made an important contribution to our study of nutrition and health.

Utah State University