PATTERN ANALYSIS OF NUTRIENT BIOMARKERS IN NEUROEPIDEMIOLOGY

By

Gene L. Bowman, N.D.

A THESIS

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CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

Gene L. Bowman, N.D.

has been approved by

Jackilen Shannon, PhD

Joseph Quinn, MD

Hiroko Dodge, PhD

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LIST OF FIGURES AND TABLES

Prologue

The thesis committee has agreed to have the candidate structure the written thesis to include an the original research conducted sandwiched between an in depth introduction and discussion that is suitable for submission to a peer-reviewed journal.

A. INTRODUCTION

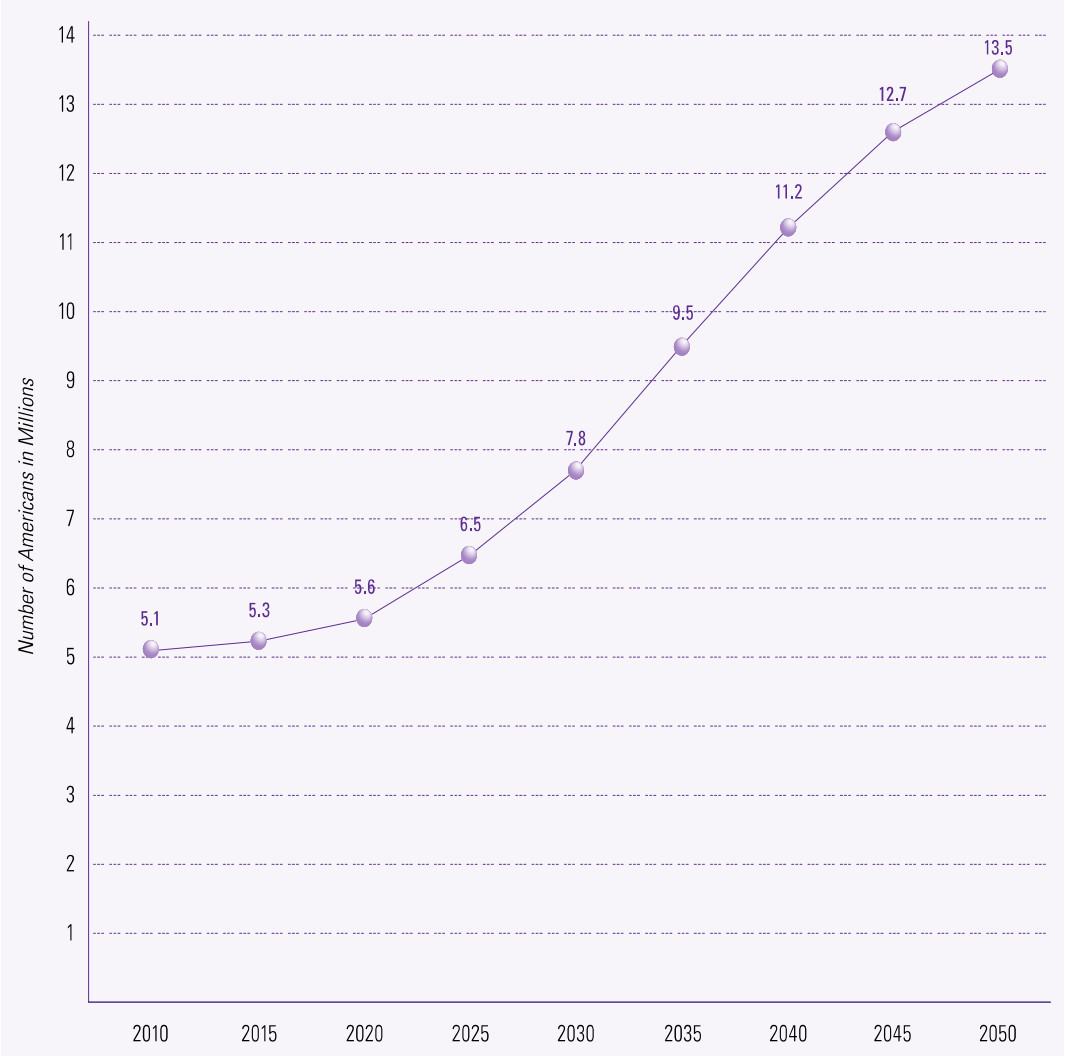
Alzheimer's disease (AD) is a devastating neurodegenerative disease of the brain that is irreversible. It has an insidious progression that destroys memory and other thinking skills severe enough to eventually affect functional capacity and the ability to carry out even the simplest activities of daily living.[1]

AGING AND ALZHEIMER'S DISEASE EPIDEMIOLOGY

The 20-year increase in average life span over the second half of the 20th century and the elevated fertility in many countries following the 2 decades after World War II will result in a larger proportion of people aged 65 years and older spanning from today to 2030.[1] This age strata (≥ 65 years) will increase from about 35 million in 2000 to 71 million in 2030 (almost 20% of the total population by 2030).[2] The prevalence of Alzheimer's disease (AD) in Americans 65 years and older is 5.1 million, and the incidence will increase dramatically over the next 40 years without the development of successful intervention (**Figure 1**). The primary risk factor is age, which is disconcerting given that the fastest growing age strata in the U.S. are those people reaching their 85th and their 65th ("baby boomers") birthday beginning in 2011 according to the U.S. Census Bureau. In fact, the prevalence of AD is projected to double for each 5-year age interval beyond

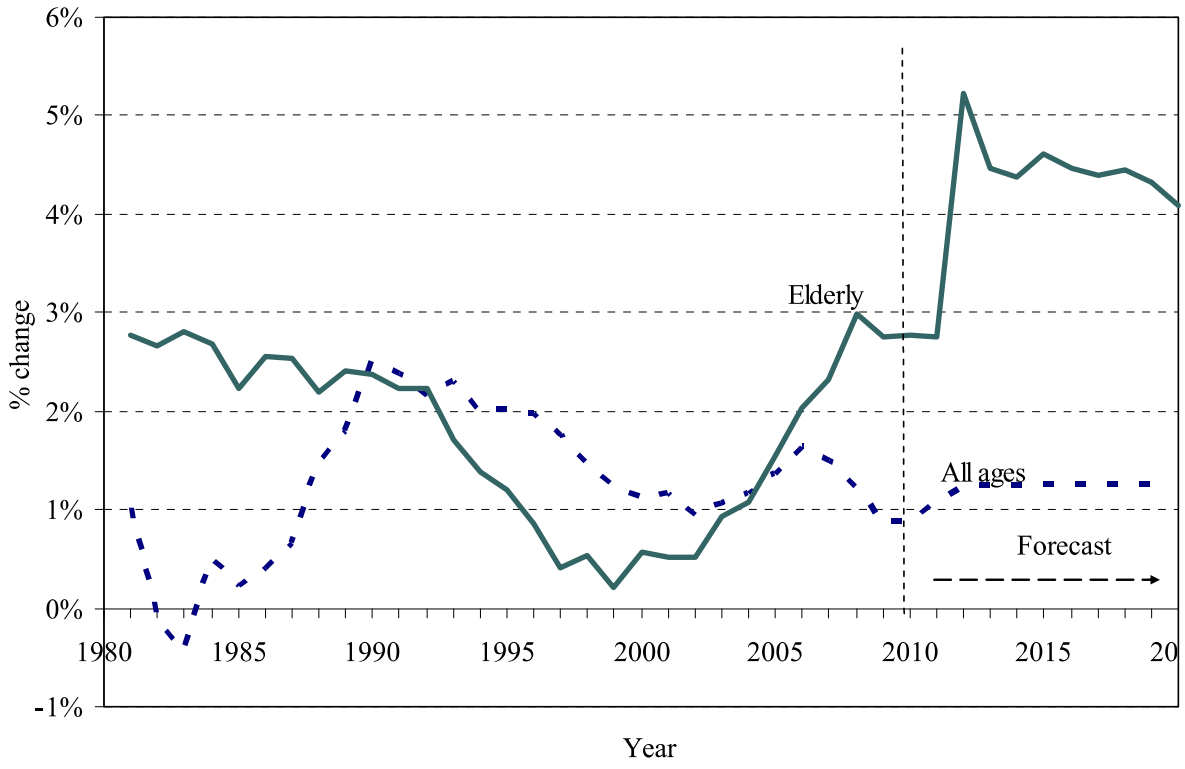
age 65. Since 2005, the growth of the elderly population in Oregon has outpaced the overall population growth due to cohort changes and the migration of older retirees to the state (**Figure 2**). The "baby-boomer" effect in Oregon will produce a growth rate in the number of elderly exceeding 5% between 2011 and 2012 and maintain above 4% growth through 2020. This will lead to the state accumulating 53% more elderly in 2020 compared to 2010 essentially placing many more people at risk for AD by virtue of age alone. These national and state epidemiologic transitions combined with the stress on caregivers of AD patients, and absence of successful preventative strategies, build justification for a major public health concern.

Figure 1. Current and Projected Prevalence of Alzheimer's disease in Americans Aged 65 and Older, 2010-2050[3]



Graph made available by the Alzheimer's Association using previously published data sources. Current prevalence and future projections for people < 65 years old is not available because that data does not currently exist.

Figure 2. Annual Rate of Change: Elderly and People of All Ages in Oregon [4]

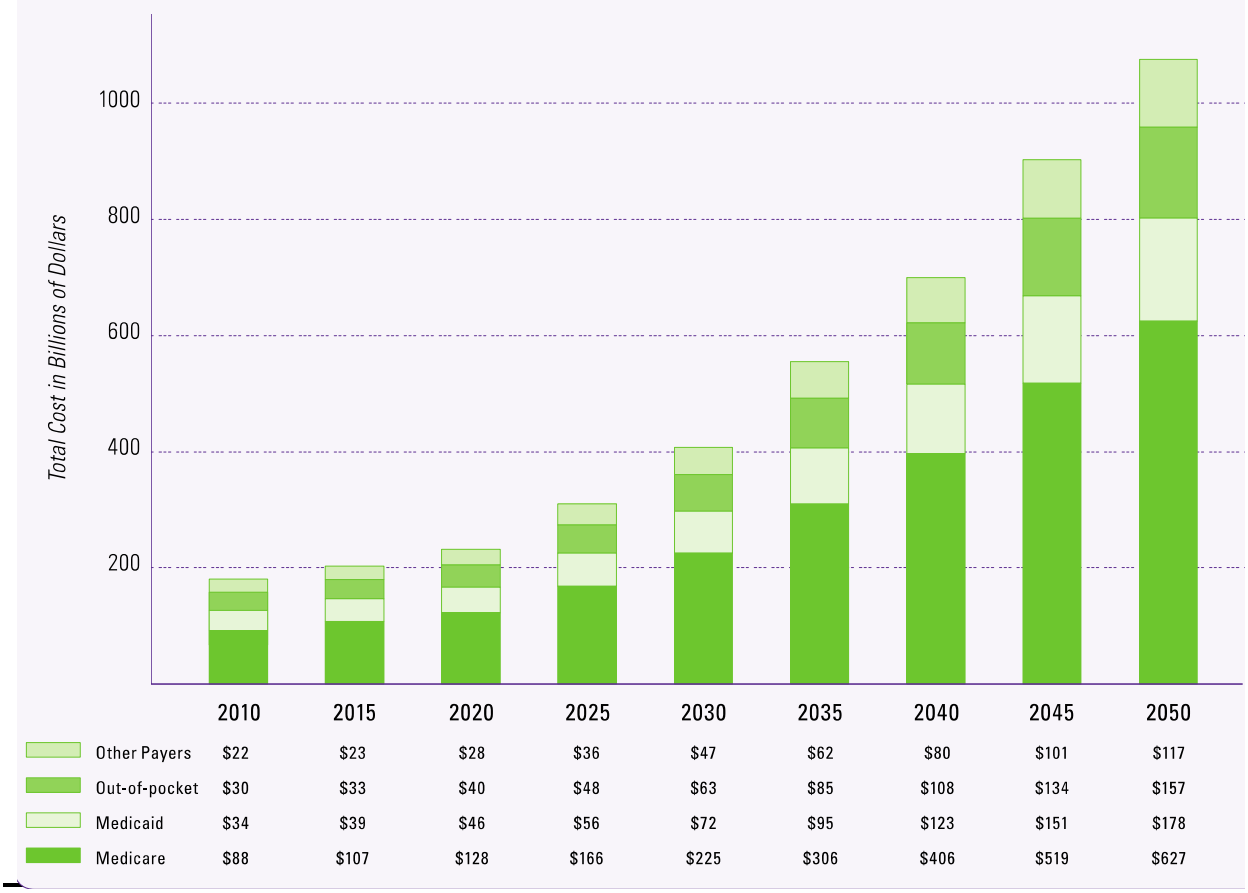


FINANCIAL IMPACT OF ALZHEIMERMER'S DISEASE

The annual cost of care for individuals with AD and other dementias age 65 years and older is currently estimated at 172 billion dollars. This does not include 12.5 billion hours of unpaid care provided by family caregiving, a role worth $144 billion to the U.S. health care system according to a 2009 report.[1] By 2050, the incidence of AD and other dementias are projected to increase five-fold. This will raise the total cost for all payers to over a trillion dollars. Medicare beneficiaries 65 years and older with AD have three times higher total cost in care compared to same age without AD ($15,145 versus $5,272) (Harting, 2009). Medicaid is also used highly by people with AD to cover nursing home and long-term care services when income and assets are deplete. AD patients over the age of 65 had Medicaid expenses nine times higher than beneficiaries in the same age

group without dementia ($6,605 versus $718) (Harting, 2009). Medicare and Medicaid costs will bloom 600% and 400%, respectively, and the out of pocket expenses will increase from 30 billion to 157 billion by 2050 (**Figure 3**).

Figure 3. Estimated Cost of Care for Americans Age 65 and Older with Alzheimer's disease and Other Dementia, 2010-2050[3]



PATHOGENESIS OF ALZHEMER'S DISEASE

Dr. Alois Alzheimer first characterized the neuropathology of Alzheimer's disease (AD) using a special staining technique in a woman who died with memory, language, and behavioral issues in 1906. During autopsy he recognized unusual "clumps" and "tangles" of fibers in her brain tissue. Today, these extracellular clumps and intracellular tangles are known as beta-amyloid plaques and neurofibrillary tangles, the prime

neuropathological features of AD. Accumulation of these plaques and tangles lead to "gapping" at the synapse, which arguably correlates best with the cognitive deficits observed clinically.

Although these pathological hallmarks of Alzheimer's disease can diffusively pepper the brain tissue, the ordinal accumulation may begin in the entorhinal cortex and hippocampus and spread to the frontal, temporal, parietal and occipital lobes. Thus, a usual case of Alzheimer's disease reports memory deterioration as the first symptom followed by other cognitive deficits as the disease spreads throughout the cortices.

CAPTURING COGNITIVE DECLINE IN AGING

Understanding the age-related and Alzheimer's disease (AD)-related cognitive decline with special emphasis on the oldest old has been actively pursued in our research at the Aging and Alzheimer's Center at OHSU. This work has centered on the Oregon Brain Aging Study (OBAS). The healthy elders in OBAS have had annual standardized clinical neuropsychological testing which has included: the Wechsler Memory Scale - Revised, the Wechsler Adult Intelligence Scale - Revised, the Boston Naming Test; Category fluency; a modified CERAD battery, and the Cognitive Competency Test. The initial analysis of cognitive function in the first 51 subjects enrolled was able to stratify the population into young elderly (mean age 69.9) and old elderly (mean age 88.8) to study differences by pure aging [5]. While there was a tendency for most tasks other than Digit Span to decline with aging, the differences were greater in visual *perceptual* and *construction tasks* than for *memory tasks*.

A recent longitudinal follow-up analysis of the cohort who have remained clinically non-demented over a four year period has shown that although there are age group differences when examined across a twenty year age span, year-to-year changes are very small.[6] For example, the non-demented oldest old lost a mean of only 0.24 points per year on a CERAD delayed word list recall task. By way of comparison, those oldest old with Clinical Dementia Rating (CDR) scale scores of 0.5 lose on average 2 points per year.

Further confirming these longitudinal analyses, the Center data demonstrate that tests of delayed recall (Logical Memory II or LM-II from the Wechsler Memory Scale - Revised and CERAD Word List Memory) are excellent predictors of later dementia. For example, scores on LM-II at entry predicted the development of transition to CDR 0.5 (conversion to Mild Cognitive Impairment) within three years with an accuracy of 75%.[7] In addition, the rate of change of scores on LM-II also predicted those that have become demented.

BRAIN STRUCTURE CHANGES AND COGNTIVE DECLINE IN AGING

Neuropathologic data is inherently cross-sectional, so that limits the inferences made regarding cognitively intact or MCI subjects with Alzheimer (AD) lesions found at autopsy as people that were likely to have become demented. Thus, the application of other corroborative approaches to address this issue is needed. This is critically important for primary prevention trials and studies of treatments that propose to show a disease altering affect. One would like to be able to monitor the loss of neurons that characterizes AD. As the clinical onset of AD is insidious, this approach would predict

that those who are losing neurons as a result of accumulating AD pathology would demonstrate neuronal loss in areas of the brain important to memory (hippocampal volume) prior to becoming symptomatic. This prediction has been substantiated in the Center's OBAS cohort of Non-Impaired Elderly where the reduction in hippocampal volume assessed quantitatively with MRI (which is significantly correlated with post-mortem neuron number; correlation coefficient ($r = 0.67$), significantly predicts those that will develop incident cognitive impairment within 3 years of the MRI [8].

Brain Volume Change in the Elderly

The Layton Center data at OHSU suggests there is very limited loss of brain volume in the oldest old who maintain cognitive health [9]. Quantitative volumetric MRI was performed annually on 46 subjects followed for a mean of 6 years without dementia. When the young elderly (< 85 years) were compared to the oldest old in this group, there was no significant difference in rate of brain volume loss in any of 13 regions of interest including the hippocampus and temporal lobes. Thus, among the oldest old who maintain their cognitive function only very minor losses of brain volume are seen and these rates of loss in non-cognitively impaired oldest old are not significantly different from those 20 years younger. These data serve as a scale for exploring whether nutrition can explain a significant proportion of this observation.

Relationship Between Brain Atrophy and Cognitive Decline

Our Center has investigated how rates of cerebral volume loss may change as Non-Impaired Elderly develop dementia. The evaluation included 102 subjects studied

longitudinally to compare the rates of volume loss with MRI in three groups: those remaining non-impaired (Clinical Dementia Rating = 0), those developing incipient dementia (CDR = 0.5) and those developing mild dementia (CDR = 1). This study demonstrated that rate of volume loss accelerates as the disease progresses and that early volume changes occur both in the medial temporal lobe structures and neocortical regions. For example, the Non-Impaired Elderly group had a negligible rate of total brain volume loss (-0.23%/yr.), as compared to the incipient dementia group (-1.11%/yr.) and the mild to moderate AD group (-2.18%/yr.). These findings support our choice to include total brain volume as a region of interest. The current study will examine the relationship between nutrient biomarker patterns and total brain volume cross sectionally and longitudinally in subjects with available MRI.

NUTRITION, COGNITIVE AGING AND ALZHEIMER DISEASE

Antioxidants, Oxidative Stress and Dementia

The low endogenous antioxidant capacity, high metabolic rate and high metal content of the brain make it inherently susceptible to oxidative stress [10]. Post-mortem studies of brain tissue consistently find oxidative damage in confirmed AD [11,12] and this is correlated to deposition of amyloid β-peptide in neuronal membranes and alteration of membrane lipid metabolism, both pathogenic factors of synaptic dysfunction and neuronal degeneration [13,14]. The possibility that the oxidative damage inherent in AD may be attenuated by antioxidant intake is supported by large observational studies that show associations between higher intake or blood levels of vitamin C, E, carotenoids, and flavonoids and protection from cognitive decline [15-17] or incident dementia {Engelhart

Morris, 20021998 #142;Maxwell, 2005 #6;Engelhart, 2002 #45;Commenges, 2000 #49;Zandi, 2004 #50}. However, antioxidant interventions have been unsuccessful in clinical trials. Two randomized, double blinded, placebo controlled trials intervening with alpha-tocopherol to slow the rate of decline in AD and Mild Cognitive Impairment have been conducted [18,19]. The trial in AD showed very modest effect of vitamin E in slowing the rate of decline, leading to the inclusion of vitamin E in practice parameters for treatment of AD. However, the magnitude of the treatment effect was so modest that some dispute any benefit of using vitamin E for the treatment of AD.[20] Enthusiasm for high dose vitamin E was diminished further by a meta-analysis indicating increased mortality in subjects receiving high dose vitamin E in a variety of clinical trials.[21] Finally, another trial that tested high dose vitamin E for MCI demonstrated no reduction in rate of progression from MCI to AD over three years in the treatment group receiving alpha-tocopherol.[19] Although results from these trials have been disappointing, it is premature to interpret these trials as evidence that antioxidant strategies are futile since the literature is abundant with evidence that suggests oxidative stress plays a pathogenic role in AD.

There are very few studies of carotenoids and cognitive function in the literature. The most compelling study was a double-blinded trial demonstrating that both lutein and DHA independently and in combination improved verbal fluency and that the combination improved memory and rate of learning.[22] An observational study in Alzheimer patients demonstrated significantly lower cerebral spinal fluid (CSF) tau proteins and higher CSF amyloid-beta 40/42 ratio in patients with higher plasma beta-carotene concentrations.[23]. Another cross sectional study demonstrated lower levels of

plasma lutein and beta-carotene in severe AD compared to mild AD and controls.[24] A larger cross sectional study of plasma carotenoids demonstrated higher odds for cognitive dysfunction in those healthy elderly with lower concentrations of lycopene and zeaxanthin.[25] In summary, very few studies have investigated the role of carotenoids in the promotion of cognitive health. However, the small clinical trial conducted by Johnson et al in healthy women is intriguing. The mechanism in which carotenoids may influence neurodegeneration is not clear. The one CSF study suggests that carotenoids may have some role in stabilizing beta amyloid and tau protein metabolism in the brain, but this data is not conclusive.

B vitamins and Dementia

Thiamin

There is a biological basis for thiamin (vitamin B1) being an important nutrient for maintaining cognitive health. Thiamin regulates key enzymes for neurotransmitter synthesis (transketolase, pyruvate dehydrogenase, and alpha-ketoglutarate dehydrogenase), modifies neuronal membrane ion pumping, and boosts neuronal energy metabolism. [26] Together with glucose and choline, thiamin is required for synthesis of acetyl coenzyme A, which leads to the production of acetylcholine.[27] Preserving this neurotransmitter in the CNS with acetyl-choline esterase inhibitors is one option for the treatment of Alzheimer's disease.[28] Thiamin is found in four different forms in humans: as free thiamin (4%), thiamin monophosphate (9%), thiamin diphosphate (84%, the active coenzyme form), and 3% as thiamin triphosphate. The triphosphate form is found

exclusively in the neuronal membranes and appears to be involved in the maintenance of the transmembrane action potential.[26]

During thiamin deficiency the triphosphate form is spared in rats, whereas free, mono- and diphosphate decrease by 20% within 4 weeks.[29] Glucose metabolism in the brain requires thiamin to convert pyruvate derived from glucose-6-phospahte to acetyl coenzyme A, which is converted to CO_2 and H_2O in reactions that require thiamin, but also riboflavin, nicotinamide, and pantothenic acid. [26]

Homocysteine and related B vitamins

The epidemiologic evidence demonstrating associations between hyperhomocysteinemia, age-related cognitive decline and dementia is abundant [30-32]. The proposed mechanisms underlying these relationships between homocysteine and cognitive performance include: 1) direct neurotoxicity, by activation of *N*-methyl-D-aspartate receptors leading to apoptosis, increased oxidative stress and increased β-amyloid toxicity [33], 2) effects of vitamin deficiency (7), and 3) vascular mechanisms including carotid artery atherosclerosis, silent brain infarction, brain atrophy, and stroke (8-11).

The first study to demonstrate that elevation of plasma homocysteine precedes dementia analyzed 1092 Framingham cohort participants' plasma homocysteine drawn between 1986-1990, prior to US folate fortification in 1998 [30]. The relative risk of AD was 1.8 (CI=1.3-2.5) per 1 SD increase at baseline, 1.6 (CI=1.2-2.1) per 1 SD increase eight years prior to baseline and a plasma homocysteine level of 14 μmol/L at baseline nearly doubled the risk for dementia. A more recently published cohort study retrospectively

analyzed plasma samples and cognitive tests recorded in 1993 as part of the Veterans Affairs Normative Aging Study [34]. This study reported decline in constructional praxis measured by spatial copying correlated inversely with plasma homocysteine, folate, vitamin B12 and vitamin B6. Further, folate remained independently protective against decline in spatial copying score after adjustment for homocysteine and other vitamins, and homocysteine correlated inversely with decline in recall memory.

Two trials of homocysteine lowering for dementia prevention have been reported, one negative and one positive. The first RCT of 276 subjects was conducted in New Zealand where folate fortification is absent {McMahon, 2006 #7}. This study reduced plasma homocysteine by about 25% (4.3 μmol/L) in the treatment group and found no significant differences on cognitive test scores over two years. There are numerous explanations for this apparent null effect including: 1) the absence of cognitive decline in the placebo group over the two years, 2) failure to achieve threshold plasma homocysteine level, and 3) failure to select a population perhaps most appropriate for this intervention (i.e., genetic mutations in enzyme catalysts of one carbon metabolism).

The second RCT of 818 subjects was conducted in a region without folate fortification as part of the Folic Acid and Carotid Intima-media Thickness Trail [35]. This primary prevention trial randomly assigned folate at 800 μg for 3 years duration observing a serum folate elevation on average of 576% and decreased plasma homocysteine of 26% (3.3 μmol/L) in the treatment group compared to placebo. At three years the treatment group demonstrated improved memory (difference in z score 0.132, 95% CI 0.032-0.233), information processing speed (0.087, 0.016-0.158), and sensorimotor speed (0.064, -

0.001-0.129) compared to placebo. An interesting quality of this study is that it applied folate therapy where it is perhaps most appropriate, in a group with elevated homocysteine, normal vitamin B12, B6, creatinine levels and exaggerated representation of a genetic mutation in the methylene tetrahydrofolate reductase (MTHFR) enzyme (46% T carriers and 18% TT homozygotes).

Vitamin D and Dementia

Vitamin D depletion is associated with cancer, immune dysfunction, and neurologic and psychological disorders aside from the well-understood impact on bone health.[36-38] Vitamin D levels < 15 ng/mL are considered a risk factor for osteoporosis according to the National Institutes of Health report in 2008, but other studies indicate a need for much higher target levels ranging from 30 ng/mL to 72 ng/mL for optimal health.[39] The current classification sets deficiency at < 20 ng/mL, insufficiency between 21-29 ng/mL and sufficient status at 30 ng/mL or greater. The ability to synthesize vitamin D from UV-B decreases with age due to the significant decline of a vitamin D precursor (7-dehydrocholesterol) in the epidermis and dermis. [39] Elders are at particular risk for vitamin D deficiency because they spend more time indoors and are less adept at synthesizing Vitamin D. The presence of vitamin D receptors in brain regions that show degeneration in AD (e.g., cortex and hippocampus), and the ability to differentiate AD subjects from normal controls based on vitamin D proteins support a plausibility for neuroprotection.[40] Very few studies have evaluated the relationship between serum 25-hydroxyvitamin D and cognitive function or Alzheimer's disease (**Table 1**).

Table 1. Epidemiological Studies of Serum Vitamin D, Cognitive Function and Risk for Dementia

Author	Design	Methods	Results
Wilkins et al [41]	Cross sectional, n=80 (40 with AD, 40 elder controls), men and women	Serum 25-OHD, cognition (Short Blessed Test, MMSE CDR-SOB) and mood (depression symptom inventory)	Lower 25-OHD assoc. with low mood and cognitive function (SBT and CDR-SOB)
Przybelski et al[42]	Cross sectional chart review, n=80, men and women	Serum 25-OHD MMSE	Positive correlation between 25-OHD and MMSE
Buell et al[43]	Cross sectional, n=318, >=65 yo men and women	Serum 25-OHD Dementia diagnosis MRI – WMH	Deficient 25-OHD assoc. OR 2.3 for all dementia Deficient 25-OHD assoc. with more WMH volume
Llewellyn et al[44]	Prospective, n=858, >=65 yo, men and women followed over a 6 year period	Serum 25-OHD MMSE, decline defined as 3 or more points Trails A and B, decline defined as worst 10% of the distribution of decline Random effects	Lower 25-OHD assoc. with cognitive decline
Slinin et al[45]	Prospective, n=1604, men followed on average 4.6 yrs.	Serum 25-OHD Logistic regression	No independent assoc. between 25-OHD with baseline 3MS or trails B, nor incident cognitive decline
Annweiler et al[46]	Cross sectional, n=752 women only >=75 yo	Serum 25-OHD Logistic regression Cognitive impairment = Pfeifer Short Portable Mental State Questionnaire < 8	25-OHD <10ng/mL assoc. with 1.9 OR of CI compared to >10 ng/mL
Llewellyn et al[47]	Cross sectional, n= 3325, >=65 yo men and women	Serum 25-OHD Logistic regression ORs Cognitive impairment = worst 10% of distribution	Increased OR of impairment per increment decrease in 25-OHD

The two prospective studies reported are mixed with one demonstrating an association of serum 25-OHD with rates of cognitive decline [44] and the other finding very little evidence

for a role of vitamin D.[45] These divergent findings may be explained by the population understudy, the different duration of follow up and cognitive outcomes employed.

Fatty acids and Dementia

Omega 3 fatty acids

Docosahexaenoic acid (DHA) is the most abundant fatty acid in the phospholipids of cerebral gray matter, representing 45-65% of total phosphatidylserine in the mitochondria [48]. Neurons of the cerebral cortex, synaptisomes, and the mitochondria are the areas of the brain with the highest metabolic activity and also highest concentration of DHA. Animal models have demonstrated that dietary intake of DHA in late life increases the DHA composition in the brain, reduces synaptic loss, preserves memory and reduces total amyloid production [48-51].

Epidemiological studies have consistently shown a significant inverse relationship between incident AD and fish consumption, a primary dietary source of DHA. Prospective studies utilizing RBC membrane fatty acid levels as a biomarker of fish consumption have also shown an inverse relationship between omega-3 fatty acid levels and cognitive decline [52]. A large study of dementia free subjects followed prospectively demonstrated a 47% lower incidence of dementia over 9 years in patients in the top quartile of plasma phosphatidylcholine DHA content (e.g., DHA level >4.2% of total fatty acids).[53]

Mechanisms by which DHA may be neuroprotective: 1) anti-inflammatory through the reduction of arachidonic acid and its metabolites via COX, lipoxygenase (PG, etc.), 2) optimizing synaptic fluidity, 3) promotion of neurogenesis, 4) reducing oxidative stress effects by increasing antioxidant enzymes (catalase, GSH peroxidase), 5) coupling blood flow to glucose utilization, 6) increasing neurotrophic factor production and signaling [54]

Consuming n-3 PUFA results in a corresponding increase of EPA and DHA in cellular and circulating lipids due to the replacement of lipids derived from n-6 PUFA such as linoleic acid and arachidonic acid. These changes appear to alter the biochemical properties of cell membranes and modify intercellular signaling. Eicosanoids such as prostaglandins, leukotrienes, and thromboxanes are mainly produced from arachidonic acid and eicosapentaenoic acid in the cell membrane by oxygenases and mediate several inter-cellular signaling processes. EPA markedly reduces thromboxane A2 by replacing arachidonic acids. This suggests that the beneficial effects of an increased intake of n-3 PUFA may be inflammatory mediated rather than by vascular means (anti-thrombotic, anti-atherosclerotic, anti-arrhythmic, antihypertensive).[55] However, this is far from conclusive.

One large epidemiological study has reported EPA as an independent predictor of dementia further supporting a role for this omega 3 fatty acid in the prevention of dementia.[56] A RCT described the effects of dietary omega 3 fatty acid supplementation on cognitive function in patients with mild-to-moderate AD.[57] It included 174 patients (mean age 74 years) with a Mini Mental Status Exam $\geq$ 15 randomly assigned to either

daily intake of 1.7 g of docosapentaenoic acid and 0.6 g eicosapentaenoic acid or a placebo for 6 months, after which all participants received the omega-3 fatty acids for 6 additional months. There were no differences in primary endpoints (Mini-Mental Status Exam or Alzheimer's Disease Assessment Scale-cog) between the two groups at six months although a subgroup with MMSE > 27 (N=32) demonstrated significant preserved MMSE score compared to the placebo group. This trial suggests that omega 3 fatty acid interventions are most effective in very early stages of neurodegeneration. A recent trial in AD was unsuccessful in slowing the progression of AD.[58] However, a secondary analysis in that trial suggested that apolipoprotein E e4 non-carriers treated with DHA may benefit. Those findings need to be confirmed in the future, but highlight more complex gene-nutrient interactions that may need to be considered in clinical trial methodology.

Trans fatty acids

Higher trans fatty acid intake has been associated with cardiovascular disease[59,60], systemic inflammation[61], and endothelial dysfunction[62,63], all of which may affect risk for AD. Currently, there are very few studies that have investigated dietary trans fat and risk for AD[64] or cognitive decline.[65,66] and no studies have described the relationship between trans fat concentrations in plasma / serum related to cognition in elders. A single study demonstrated that trans fat may replace neuronal DHA, but that pathological hallmarks of AD are not affected.[67] Another hypothesis with some early support suggests that dietary trans fat may aggravate cognitive function both independently and synergistically through interaction with copper intake.[68]

Cholesterol, 24S-Hydroxycholesterol and Dementia

Cholesterol is the main lipid constituent of neuronal membranes and myelin, synthesized in the brain in situ and independent of extra-cerebral cholesterol.[69] Brain cholesterol is mostly independent from dietary intake and nearly completely synthesized de novo in the brain.[70] The blood-brain barrier (BBB) prevents transport of cholesterol from the brain to the periphery, thus another mechanism is required that may be mediated by apolipoprotein E and facilitated by 24S-hydroxycholesterol (24S-OHC).[71] 24S-OHC is a brain-specific oxysterol, which readily crosses the BBB and may reflect brain cholesterol homeostasis more closely than plasma total cholesterol. In fact it is reported that 90% of plasma 24S-OHC levels originate in the brain [71], and the concentration gradient favors diffusion from the CSF to the periphery. Reduced absolute plasma 24S-OHC is suggested as a marker of neuronal degeneration [72]. CSF studies of AD and Mild Cognitive Impairment have shown elevated levels of 24S-OHC suggesting that elevation of 24S-OHC occurs early in the disease process serving as a potential marker for monitoring onset and progression of the disease.[73] Increased CSF and decreased plasma 24S-OHC reported in AD compared to healthy controls suggest reduced clearance of cholesterol from the brain to the periphery through the blood brain barrier in these patients.[72-74] Plasma 24S-hydroxycholesterol studies in dementia are mixed [73-76] This may be explained by reduction of plasma 24S-hydroxycholesterol with niacin (vitamin B3) and statin treatment, not sufficiently controlled for in these analyses [77,78,79] and the need to correct for peripheral cholesterol levels. Since plasma 24S-OHC and cholesterol are highly correlated {Bretillon, 2000 #6 24S-OHC should be corrected for plasma cholesterol and reported as a ratio. Deflated plasma

ratio of 24S-hydroxycholesterol-to-total cholesterol is seen in subjects with AD and MCI compared to controls.[80]

ADVANCING THE STUDY OF NUTRITION IN COGNTIVE AGING AND ALZHEIMER'S DISEASE

The study of nutrition in population health has historically utilized dietary surveys for exploring the relationship between diet and disease. This work has helped identify prudent diets for chronic diseases, but many inconsistencies exist and the attempts to translate these findings into successful clinical trials have remained futile. The explanation for these inconsistencies require an understanding of epidemiological and biostatistical methods, clinical trial methodology and a good foundation in the biology of the disease of interest. The opportunity to advance the study of nutrition for neurodegenerative disease is robust because the conceptual and methodological issues in the field are pronounced. There are two areas of opportunity to focus the advancement regarding diet and dementia: 1) improving the classification of dietary exposure in older populations using biological markers of diet, and 2) establishing a conceptual framework better suited for describing how nutrition affects brain aging. Regarding the first area, it is accepted that there is a certain level of information bias inherent to reporting of diet with dietary surveys. However, there is common misconception that misclassification bias of nutritional status can only attenuate the measure of association. When the miss-classification bias is different between the cases destined to develop Alzheimer's disease (more memory impairment equals more misclassification of dietary exposure) compared to those dementia-free over time, the measure of association can be overestimated. This

is a form of differential misclassification bias. This methodological fact may contribute to the inconsistencies in dietary studies utilizing food questionnaire methods in older populations. The second opportunity for advancing the field is a movement from emphasis on single or few nutrients to food or nutrient combinations. This approach is a better conceptual fit for the nature in which nutrients behave biologically and how they are consumed and observed in our environment. Disappointing clinical trials on the single or few nutrient approach for AD or cognitive decline have failed (i.e. vitamin E, homocysteine, DHA). Now that we have good data to support the ineffectiveness of nutrients in isolation for neurodegenerative disease, we can search for rigorous means to better understand nutrients in combination, their interactive features, and how they collectively influence chronic disease. This is the main thrust of this thesis.

Available Methods For Studying Nutrient / Food Combinations: The Combo Approach

Over the past decade, existing methods have been adopted to permit the study of dietary patterns. A collection of nutrients representing a "pattern" of nutritional status or intake can be regressed on an outcome. These methods are described as pattern analysis. Factor and cluster analysis and dietary indices are some examples of techniques that characterize nutrient/food combinations. Factor and cluster analysis have been considered *a posteriori* techniques because the patterns are derived from statistical modeling of the data at hand.[81] One could argue that factor analysis combines existing knowledge and the data at hand to generate the patterns when the methods and variables included in the model are clearly stated *a priori*. The dietary indices approach is "*a priori*" because the

indices are constructed based on present knowledge about the nutrient-disease relationship. Factor and cluster analysis are multivariate statistical techniques that are slightly different by how they generate factors and clusters.

The most common variety of factor analysis is principal component analysis (PCA). This procedure recognizes common underlying dimensions in constructing patterns sometimes called factors, components, or patterns.[82] PCA assembles the patterns on the basis of intercorrelation among the variables entered into the model. Each pattern derived is orthogonal (perpendicular from one another) essentially creating independent patterns. Summary scores are derived for each participant, which are linear combinations of each nutrient in each distinct pattern. The patterns derived are usually standardized and treated as a continuous variable. Once the procedure is complete, regression models can be fit with the principal components as predictors of outcomes of interest.

Cluster analysis is another data driven multivariate method for characterizing nutrient patterns. This method aggregates individuals into relatively homogenous clusters of dietary habits. For example, subjects eating high fruits and vegetables would be placed into one cluster while those consuming high trans fat and red meat and low fruits and vegetables would be placed in another exclusive cluster.

Dietary indices are constructed on basis of previous knowledge. These have been used to determine a subject's adherence to dietary guidelines considered "healthy" or "unhealthy" and how that associated with disease. Mediterranean diet adherence score is another

example of a dietary indices approach. In this example, subjects can be judged on 9 dietary constituents that are characteristics of the Mediterranean diet. An intake below or above the median for the population under study decides the binary score, 0 or 1. The investigator needs to score the favorable and unfavorable dietary characteristics correctly to maintain directionality. For example, high red meat and low vegetable intake would receive the same score of zero.

There are several reasons for the evolution of dietary studies to move in this direction: 1) clinical trials testing a single or few nutrients as a panacea of good health have failed consistently [19,58,83-87]; 2) focusing on single or few nutrients is far from the manner in which people consume food. Our diets include complex combinations of nutrients and foods and these observations may offer clues into which nutrients work best in combination. The observation that iron absorption is enhanced in the presence of vitamin C{Health, 1989 #716} would go undetected without collection of vitamin C in a study of iron; 3) intercorrelation among some nutrients (such as iron and vitamin C, magnesium and potassium, DHA and EPA, folate and vitamin B12) makes it difficult to examine their separate effects because the degree of independent variation of the nutrients is markedly reduced when they are entered simultaneously into a model[88]; 4) nutrients in isolation are likely to have minimal effects, too small to detect under the constraints of feasible study designs. This is particularly important in the case of chronic diseases where the development is insidious requiring large sample sizes. There is good evidence to support a more robust effect of nutrient combinations for chronic disease [89-91]; 5) an analysis based on a large number of individual nutrients or food items may produce

associations too frequently by chance alone[92]; 6) dietary patterns in the population may confound any single nutrient effects.[93-95] **Table 2** highlights epidemiological studies of dietary patterns using food frequency questionnaires and results related to AD and cognitive decline.

Table 2. Epidemiological Studies Of Dietary Patterns, Cognitive Function, and Dementia

Author	Design	Methods	Results
Scarmeas et al[96]	Prospective of 2258 non demented elders with a mean follow up of 4 years	Dietary index of Mediterranean diet using FFQ; scored individuals on the basis of beneficial and detrimental food consumption (median cutoff 0 or 1) Nine categories: fruits vegetables, legumes, cereals, fish, meat, dairy, fat, alcohol	Higher adherence to the MeDi was associated with lower risk for AD (HR, 0.91; 95% CI, 0.83-0.98; p=0.015). Compared with subjects in the lowest MeDi tertile, subjects in the middle MeDi tertile had a hazard ratio of 0.85 (95% CI, 0.63-1.16) and those at the highest tertile had a hazard ratio of 0.60 (95% CI, 0.42-0.87) for AD (p=0.007).
Barberger-Gateau et al[97]	Prospective study of 8,085 non-demented elders >=65 years old followed for mean of 4 years participating in the Three-City cohort study in Bordeaux, Dijon, and Montpellier (France)	Built a model considered brain healthy using FFQ based on existing knowledge and significant univariate relationships with the cognitive outcome.	Daily consumption of **fruits** and **vegetables** was associated with a decreased risk of all cause dementia (HR, 0.72, 95% CI 0.53, 0.97) -Weekly consumption of **fish** was associated with a reduced risk of AD (HR 0.65, 95% CI 0.43 to 0.994) and all cause dementia but **only among ApoE ε4 non-carriers** (HR 0.60, 95% CI 0.40 to 0.90) -Regular consumption of **omega-6 rich oils** not compensated by consumption of omega-3 rich oils or fish was associated with an increased risk of dementia (HR 2.12, 95% CI 1.30 to 3.46) **among ApoE ε4 non-carriers only**
Samieri et al[98]	Cross-sectional study of 1,724 elders living in Bordeaux, France from 2001 to 2002.	Cluster analysis using FFQ	Five dietary clusters were identified in each sex. A "healthy" cluster characterized by higher consumption of **fish** in men (n=157; 24.3%) and **fruits** and **vegetables** in women (n=267; 24.8%) had significantly lower mean number of errors to MMSE score (beta=-0.11; 95% CI, -0.22 to -0.004 in men; beta=-0.13; 95% CI, -0.22 to -0.04 in women).
Scarmeas	Prospective study of	Dietary index of	-Compared with subjects in the lowest

et al[99]	1393 cognitively intact elders followed on average of 4.5 years	Mediterranean diet using FFQ	MeDi adherence tertile, subjects in the middle tertile had 17% less risk ([HR] = 0.83; 95% CI, 0.62-1.12; P = .24) of developing MCI and those in the highest tertile had 28% less risk (HR = 0.72; 95% CI, 0.52-1.00; P = .05) of developing MCI (trend HR = 0.85; 95% CI, 0.72-1.00; P for trend = .05). -Compared with subjects in the lowest MeDi adherence tertile, subjects in the middle tertile had 45% less risk (HR = 0.55; 95% CI, 0.34-0.90; P = .01) of developing AD . Those in the highest tertile had 48% less risk (HR = 0.52; 95% CI, 0.30-0.91; P = .02) of developing AD (trend HR = 0.71; 95% CI, 0.53-0.95; P for trend = .02).
Gu et al[100]	Prospective study of 2148 elders >=65 yo free of dementia followed on average 3.9 years	Reduced rank regression Using FFQ and informed by mediating biomarkers	A dietary pattern represented by higher intakes of **salad dressing**, **nuts, fish, tomatoes, poultry**, **cruciferous vegetables, fruits,** and **dark and green leafy vegetables** and a lower intake of **high-fat dairy products, red meat, organ meat, and butter** strongly associated with lower AD risk: compared with subjects in the lowest tertile of adherence to this DP, the AD hazard ratio (95% confidence interval) for subjects in the highest DP tertile was 0.62 (0.43-0.89) (*P* = .01)
Tangney et al[101]	Prospective study of 3790 (2280 blacks, 1510 whites) with mean follow up of 7.6 years	Dietary index comparing adherence to MeDi diet versus Healthy Eating Index (HEI)	Higher MeDi scores were associated with slower rates of cognitive decline (beta = +0.0014 per 1-point increase, SEE = 0.0004, P = 0.0004). No such associations were observed for HEI-2005 scores.

Food frequency questionnaires have traditionally been used to assess dietary patterns in relation to disease, [102] but the information bias inherent to these methods may be exaggerated in older populations. Biological markers of diet are reproducible and validated in subjects at risk for dementia and offer an alternative strategy for dietary assessment in older populations.[103] To our knowledge, there have been no studies applying pattern analysis to a diverse panel of nutrient biomarkers. For these reasons, the thesis will pursue the following specific aims:

B. THESIS SPECIFIC AIMS:

The thesis aim is to apply pattern analysis to a set of biological markers of diet in hopes of establishing a new avenue for the study of nutrition and dementia. This thesis is that this approach may provide a nutritional therapy more capable of affecting a chronic disease through an appreciation of nutrient synergies. The ultimate goal is to define an optimal dietary pattern for promoting cognitive health to reduce the incidence of Alzheimer's disease. Three specific aims were pursued:

AIM 1: Construct and examine the structure of the nutrient biomarker patterns in the Oregon Brain Aging Study.

AIM 2: Cross-sectional study: Determine the association between Nutrient Biomarker Patterns and Cognitive Function and Brain Volume. *Hypothesis: NBPs are associated with cognitive function/brain volume.*

AIM 3: Prospective study: Determine the association between Nutrient Biomarker Patterns and Rates of Change in Cognition. *Hypothesis: NBPs are associated with cognitive change score.*

C. MANUSCRIPT DEVELOPMENT

Nutrient Biomarker Patterns, Cognitive Function and MRI Measures of Brain Aging

Gene L. Bowman, ND[1, 2], Lisa C. Silbert, MD[1], Diane Howieson, PhD[1], Hiroko Dodge, PhD[1], Maret Traber, PhD[3], Balz Frei, PhD[3], Jeffrey A. Kaye, MD[1], Jackilen Shannon, PhD[2], Joseph F. Quinn, MD[1]

[1]Department of Neurology, [2]Department of Public Health and Preventive Medicine, Oregon Health & Science University, Portland; [3]Linus Pauling Institute, Oregon State University, Corvallis.

ABSTRACT

BACKGROUND. Single or few nutrients to slow the progression or delay the onset of Alzheimer's disease have been unsuccessful. The minimal effect that nutrients have in isolation may explain these negative results. A specific nutrient combination may produce more robust effects through synergistic qualities. However, rigorous means for defining an ideal combination for the promotion of cognitive health have remained elusive. We identified distinct nutrient biomarker patterns in a population at risk for dementia and examined their relationship with cognitive and neuroimaging outcomes.

METHODS. A diverse set of 30 nutrient biomarkers were analyzed in plasma from 104 elders at risk for dementia. Data driven multivariate analysis constructed nutrient biomarker patterns and regression models tested their relationship cognitive and neuroimaging parameters.

RESULTS. Mean age was 87 ±10 and 62% were female. Eight distinct nutrient biomarker patterns (NBP1-8) were identified and three of these were associated with both cognitive and neuroimaging outcomes; two favorable (NBP1 and NBP5) and one unfavorable (NBP8). These results were independent of age, gender, education years and global cognitive state. In prospective analysis, NBP1 and NBP8 were associated with slower and faster rates of cognitive decline, respectively.

CONCLUSION. These findings support the use of biological markers of diet in older populations at risk for dementia, and the application of data driven methods to derive nutrient biomarker patterns with synergistic features. This approach may yield a nutritional therapy more capable of promoting cognitive health in people at risk for dementia.

INTRODUCTION. The epidemiology of Alzheimer's disease (AD) suggests a role for nutritional factors in the development of this most common form of dementia.[17,53,65,97,104-106] Despite the studies in favor of single or few nutrients for AD prevention, the translation of the single or few nutrient approach using vitamin E, B vitamins (vitamin B6, folate and B12), or docosahexaenoic acid in formal clinical trials have been unsuccessful.[19,58,87] Given the interactive nature of nutrient action and metabolism, it is not surprising that the single or few nutrient approaches to modify a neurodegenerative disease is tenuous.[83-85] Recent results provide strong rationale for revising our methods to better understand nutrients in combination, their interactive features, and how they may collectively influence cognitive performance.

The study of dietary patterns offers a broad view of the nutritional exposure and can identify important interactions or synergy among foods and their constituents. Food frequency questionnaires (FFQ) have traditionally been used to construct dietary patterns.[102] While FFQ data is relatively inexpensive and fairly comprehensive, this method fails to account for the effect of memory impairment upon recall of dietary intake or for variability in absorption of nutrients, both of which are common in elderly populations.[107,108] We have recently reported superior reliability and validity of biological markers of diet in elders at risk for dementia.[109] In the current study, we examine the candidacy of these nutrient biomarkers in explaining cognitive and MRI measures of brain aging.

In an effort to capture the effect of nutrients in combination, rather than focus on single nutrients, we construct nutrient biomarker patterns using principal component analysis. Cluster analysis[98], dietary indices[96], and reduced rank regression[100] have each been applied to food questionnaire data to assemble dietary patterns and their association with cognitive decline, but these methods have not been applied to an objective panel of nutrient biomarkers. The goal is to define an optimal dietary pattern for promoting cognitive health, in the same manner that optimal dietary patterns for controlling hypertension have been successfully derived and applied.[90]

METHODS. *Study Population.* The Oregon Brain Aging Study (OBAS) was initiated in 1989 and has been described in detail previously. The Institutional Review Board for human study at Oregon Health and Science University approved all procedures, including plasma banking. This study originally recruited community dwelling elder men and women aged 65 years and older with no history of vascular disease or risk factors and other comorbidities that were thought to affect cognition at that time. Cognitive status was based on the Mini Mental State Examination (MMSE), neuropsychological assessment, and the Clinical Dementia Rating (CDR). Only non-demented, non-impaired subjects were enrolled. Each participant attends an annual study visit with a collateral historian for evaluation by a staff neurologist, neuropsychologist and research study member for clinical and cognitive evaluation, MRI and blood collection. Plasma banked during the 2006-2007 circa was used for this cross sectional study. Clinical and neuropsychological data was available for 104 subjects and MRI data was available for

42 participants. Participation was confined to active OBAS participants with a CDR of 0 or 0.5 at the time of the blood draw.

Nutrient Biomarker Acquisition And Analysis. Plasma was originally collected and banked between the hours of 0700 and 1200 noon Pacific Time beginning in September of 2006 and ending December 2007. The samples were assayed for the following analytes: Heparin plasma was deproteinized with 10% metaphosphoric acid and analyzed for ascorbic acid using HPLC.[110] EDTA plasma carotenoids, tocopherol, and retinol were analyzed by HPLC using diode array detector and fluorescence detector.[111] EDTA plasma thiamin, riboflavin, niacin, and pyrodoxal 5-phoshate (B6) were analyzed by LC-MS/MS.[111] EDTA plasma folate and vitamin B12 were measured with a chemiluminescence-based assay on an Immulite analyzer (Siemens Corporation; Washington DC). RIA was used to measure EDTA plasma 25-OH vitamin D (Immunodiagnostics Systems Inc.; Scottsdale AZ). Absolute plasma fatty acid concentrations were quantified using gas chromatography equipped with a flame ionization detector.[111] Enzymatic methods quantified plasma total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol. Reliability statistics for these assays are published.[109]

Neuropsychological Test Battery. The cognitive testing administered annually in the Oregon Brain Aging Study is systematic and comprehensive.[5] These assessments include the Mini-Mental State Examination (MMSE), the Clinical Dementia Rating scale, Digit Span Forward and Backwards, Trails Making Test - Part A and B, Consortium to Establish a Registry for Alzheimer's Disease word list acquisition and delayed recall, the

Boston Naming Test, and the Wechsler Adult Intelligence Scale – Revised Logical Memory IA, IIA and Block Design.

Volumetric Brain MRI Acquisition And Analysis. MRI scans in the Oregon Brian Aging Study are performed with a 1.5 T magnet.[8] REGION image analysis software quantified regional brain volumes of interest. Recursive regression analysis of bi-feature space based on relative tissue intensities was used to separate tissue types on each coronal image. The sums of pixel areas for all slices were converted to volumetric measures by multiplying by the slice thickness for each of the following regions of interest: total white matter hyperintensity volume (WMH, includes periventricular and subcortical deep signals) and supratentorial brain volume as total cerebral brain volume (TCBV, excluding cerebellum and brain stem). Intracranial volume (ICV) was determined by automatically regressing for brain tissue, CSF, and WMH collectively against bone, creating a boundary along the inner table of the skull. Additional boundaries were manually traced along the tentorium cerebelli and the superior border of the superior colliculus, the pons, and the fourth ventricle. The pituitary, vessels in the sphenoid region, and any sinuses that may have been included by the automatic regression were also excluded manually. Intraclass correlation coefficients for the volumetric MRI methods were 0.95 for all regions except WMH volume (ICC = 0.85).

Covariate Evaluation. Genotyping of apolipoprotein E was determined by polymerase-chain-reaction assay.[112] Baseline interviews were queried for age, gender, education, body mass index (BMI) (weight in kilograms divided by height in meters squared), socioeconomic status, blood pressure, diabetes, smoking and drinking status, multivitamin use, fasting duration prior to blood draw, and plasma creatinine. Presence

of comorbidity required current treatment for the condition. Socioeconomic status was determined using Hollingshead's two-factor model.[113] Geriatric Depression Scale[114]and Hachinski Ischemic Score[115] were also collected at baseline to assess depression and vascular burden, respectively.

Statistical Analysis. Cognitive *Z*-scores were generated on conceptual basis[116] to represent cognitive domains (**table 3)**. MRI derived total cerebral brain volume was expressed as the % of total intra-cranial volume to adjust for head size. White matter hyperintensity volume included both periventricular and subcortical deep signals and was expressed as a % of total cerebral brain volume to adjust for brain size. Data driven multivariate analysis (principal component analysis, PCA) generated distinct nutrient biomarker patterns. Eigenvalue was set *a priori* at > 1.0 to determine the nutrient biomarker patterns to carry into hypothesis testing. Linear regression models were fit with nutrient biomarker patterns as independent predictors of cognitive z-scores and volumetric MRI while adjusting for important covariates for the cross sectional analysis. The longitudinal analysis employed a change score analysis fitting a multivariate linear regression model to test the association between nutrient biomarker patterns and annual rates of change in cognition the Clinical Dementia Rating – sum of boxes score by the following equations: CDR-SOB change = (CDR-SOB follow up – CDR-SOB at baseline)/time interval (years). IBM SPSS version 18 statistical software was utilized to conduct the analyses with two-tailed *P* values and α level set at 0.05 to denote significance.

Table 3. Construction of the Cognitive Z-scores

Attention = $(Z_{\text{Digit Span Forward}} + Z_{\text{Digit Span Backwards}}) / 2$
Executive function = $(Z_{\text{Trail Making Test B}})$
Language = $(Z_{\text{Animal List}} + Z_{\text{Vegetable List}} + Z_{\text{Boston Naming Test}}) / 3$
Memory = $(Z_{\text{WMSR LM IA Immediate}} + Z_{\text{WMS LM IIA Delayed}} + Z_{\text{CERAD Word List Immediate}} + Z_{\text{CERAD Word List Delayed}}) / 4$
Processing speed = $(Z_{\text{Trail Making Test A}})$
Visuospatial function = $(Z_{\text{WMS-R Block Design}})$

RESULTS

Study Participant Characteristics

Table 4. Demographic and Clinical Characteristics of the Oregon Brain Aging Study

		All N=104
Age, mean (SD), y		87 (10)
Female gender, No. (%)		64 (62)
Education, mean (SD), y		15 (3)
APOE4 carrier, No./total (%)		9/98 (9)
BMI, mean (SD), kg/m^2		25 (4)
BP, mean (SD), mm Hg		
Systolic		124 (17)
Diastolic		66 (10)
Creatinine, plasma, mean (SD), mg/dL		1.0 (0.3)
Hypercholesterolemia, No./total (%)		24/104 (23.1)
Smoking, No./total (%)		2/104 (2)
Drinking, No./total (%)		40/86 (39)
Diabetes, No./total (%)		5/104 (4.8)
Hypertension, No/total (%)		46/104 (44.2)
Depression, No./total (%)		22/104 (21.2)
Multivitamin use, No./total (%)		25/95 (26)
Fasting Duration, mean (SD), hours		3.3 (2.8)
Social Economic Status scale, mean (SD)		49 (10)
Hachinski ischemic scale score, mean (SD)		1.7 (2.0)
Neuropsychological Tests, mean (SD)		
Global	Mini Mental State Exam	27 (3)
	Clinical Dementia Rating	0.17 (.24)
Attention	Digit Span – Forward	6.4 (1.2)
	Digit Span - Backward	4.7 (1.2)
Executive function	Trail Making Test B	139 (79)
Language	Boston Naming Test	26 (4)
	Category fluency – Animals	17 (7)
	Category fluency – Vegetables	13 (6)
Memory	Logical Memory IA	15 (4)
	Logical Memory IIA	14 (5)
	Word-List Acquisition	19 (5)
	Word-list Recall	5.7 (2.7)
Processing speed	Trail Making Test A	44 (23)
Visual spatial	Block Design	23 (8)
Volumetric MRI, mean (SD), cm^3		***N = 42***
Total Intracranial Volume (TIV)		1164 (118)
Total Cerebral Brain Volume (TCBV)[1]		833 (89)
White Matter Hyperintensity Volume (WMH)		16 (10)
TCBV as % of TIV		72 (4)
WMH as % of TCBV		1.9 (1.2)

Abbreviations: *APOE4*, apolipoprotein E epsilon 4; MRI, Magnetic Resonance Imaging; Comorbidities require active treatment to qualify.[1]Total cerebral brain volume excludes brain stem and cerebellum

OBAS participants were white, non-Hispanic with mean college education of 3 years. The mean age was 87 ±10, 62% were women, and 9% were *APOE4* allele carriers. Smoking, diabetes, depression, and obesity were present in less that 25% of the population. Twenty-six percent of the cohort reported multivitamin supplementation and about half were currently drinking. The mean MMSE was 27 and no participants had a Clinical Dementia Rating > 0.5. Forty-two participants had MRI scans available within a month of the nutrient blood draw. The mean supratentorial brain volume (total cerebral brain volume, TCBV) was 833 ± 89 cm^3 (**table 4**).

Correlation Structure and Interpretation of Nutrient Biomarker Patterns using PCA

Table 5. Principal Component Analysis of Nutrient Biomarkers: Structure and Variance Explained[1]

Nutrient Biomarkers	Nutrient Biomarker Patterns							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Pyridoxal – 5 - phosphate (B6)	**.847**[§]				.210			
Thiamin (B1)	**.812**					-.208		-.269
Riboflavin (B2)	**.788**							.246
Folate (B9)	**.765**					.180		
Ascorbic acid (vitamin C)	**.649**		.265			.189	.264	-.180
α-Tocopherol (vitamin E)	**.631**	.277		.287				
Cobalamin (B12)	**.558**	-.208	.316	-.358		.279		
25-hydroxyvitamin D	**.555**	-.209			.343	.253		.215
α-Linolenic acid (18:3n-3)		**.866**						
Palmitic acid (16:0)		**.863**		.190	.219			
Triacylglycerol		**.841**					-.360	
Heptadecanoic acid (17:0)		**.750**			.280			
Linoleic acid (18:2n-6)		**.727**		.421		.188		
Trans - Elaidic acid (18:1n-9*t*)		**.646**	-.169	-.264	-.359			
α-Carotene			**.778**		.271		.272	
β-Cryptoxanthin			**.700**	.165			-.164	
β-Carotene	.403		**.691**		.267			
Lycopene			**.552**		-.365		.306	
LDL – Cholesterol				**.849**		.177		
Cholesterol		.246	.175	**.799**		.337	.240	
Eicosapentaenoic acid (20:5n-3)	.180		.160		**.808**	.204	.229	
Docosahexaenoic acid (22:6n-3)		.234	.185		**.804**			
Arachidonic acid (20:4n-6)				.231	.289	**.806**		
γ-Linolenic acid (18:3n-6)		.337		.192	.181	**.685**		
Retinol		.162			-.209	**.569**		.566
HDL - Cholesterol		-.239	.192	.256		.302	.669	
Lutein + Zeaxanthin			.476				.630	.222
Uric acid	-.200	.273			-.176		-.594	.261
Niacin (B3)	.208			.409			-.503	-.402
Trans - Linolelaidic acid (18:2n-6*t*)				.167		-.163		**.729**
% Variance explained by each NBP	**21**	**15**	**11**	**7**	**5.4**	**4.5**	**4.5**	**4.1**
Cumulative % of variance explained	**21**	**36**	**47**	**54**	**50**	**64**	**68**	**73**

[1]Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.
[§]Nutrient biomarker patterns are interpreted on the basis of the strongest nutrient loading coefficients within each pattern. For example, NBP1 is interpreted as a profile mostly represented by high circulating plasma B vitamins, vitamins C, E, and D (loading coefficients > 0.50). Each participant receives a summary score comprised of the linear combination of the predominant plasma nutrients within each pattern. Coefficients < 0.15 were excluded to simplify the table and bold face highlight the major nutrients within each pattern.

Principal component analysis (PCA) generated eight distinct nutrient biomarker patterns (NBPs) that met our inclusion criteria of eigenvalue > 1.0 (**table 5**). Together these NBPs explained 72.8% of the total variance present in the original set of 30 nutrient biomarkers (cumulative % variance after NBP8 extraction, **table 5**). For the sake of

reference in the following results, NBP1 is described as the plasma vitamin "BCDE" pattern (all loading coefficients > 0.50). NBP2 is described as the plasma "saturated fat" pattern, NBP3 as the "carotenoid" pattern, and NBP4 as the "cholesterol" pattern. NBP5 is described as the "marine omega 3" fatty acid pattern, and NBP6 as the "omega 6 + retinol" fatty acid pattern. NBP7 is mostly represented by lutein + HDL-cholesterol and is described as the "lutein and HDL" pattern, and NBP8 as the "trans fat" pattern mostly represented by *trans* linolelaidic acid (18:2n-6*t*).

Association of Nutrient Biomarker Patterns with Cognitive Function in OBAS

Table 6. Summary of Linear Regression Analysis: Association of Nutrient Biomarker Patterns with Cognitive Function[1]

	Cognitive Domain[2]											
	Executive		Memory		Attention		Visuospatial		Language		Process speed	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*
NBP1	-.213	.026*	.116	.164	.308	.002*	.214	.031*	.147	.077	-.165	.068
NBP2	.060	.536	.063	.463	.015	.880	-.002	.988	-.135	.118	-.105	.262
NBP3	.007	.945	-.043	.606	.181	.063	-.042	.664	.002	.983	-.075	.409
NBP4	-.120	.229	.025	.767	.042	.670	.047	.640	.016	.853	-.070	.459
NBP5	-.232	.037*	.061	.517	.017	.875	.025	.824	-.027	.774	-.125	.227
NBP6	-.137	.164	-.176	.042*	-.101	.311	-.045	.653	-.180	.039*	-.151	.107
NBP7	.067	.477	.230	.008*	.021	.828	.029	.767	.070	.407	-.051	.574
NBP8	.112	.239	-.200	.018*	-.260	.008*	-.083	.395	-.198	.018*	.208	.025*

NBP, nutrient biomarker pattern summary scores: NBP1, BCDE; NBP5, marine omega 3; NBP6, omega 6; NBP7, lutein and HDL-cholesterol; NBP8, trans fat.
[1]All analysis adjusted for age, gender and education. Significant findings are color coded: favorable green and unfavorable orange
[2]Cognitive Z-scores

Linear regression models were fit for each of the six cognitive *Z*-scores by entering the eight NBPs as independent predictors and adjusting for age, gender and education years. Three favorable (NBP1, NBP5, NBP7) and two unfavorable (NBP6 and NBP8) patterns are identified. High NBP1 (BCDE) scores associated with better attention, executive function, and visuospatial skills. High NBP5 (marine omega 3) associated with better executive function and high NBP7 (lutein and HDL-cholesterol) associated with better

memory scores. High NBP8 (trans fat) scores associated with worse attention, language, memory and processing speed and high NBP6 (omega 6 + retinol) associated with worse memory and language fluency (**table 6**).

Association of Nutrient Biomarker Patterns with MRI measures of Brain Aging in OBAS

Table 7. Summary of Linear Regression Analysis: Association of NBP with MRI Measures of Brain Aging[1]

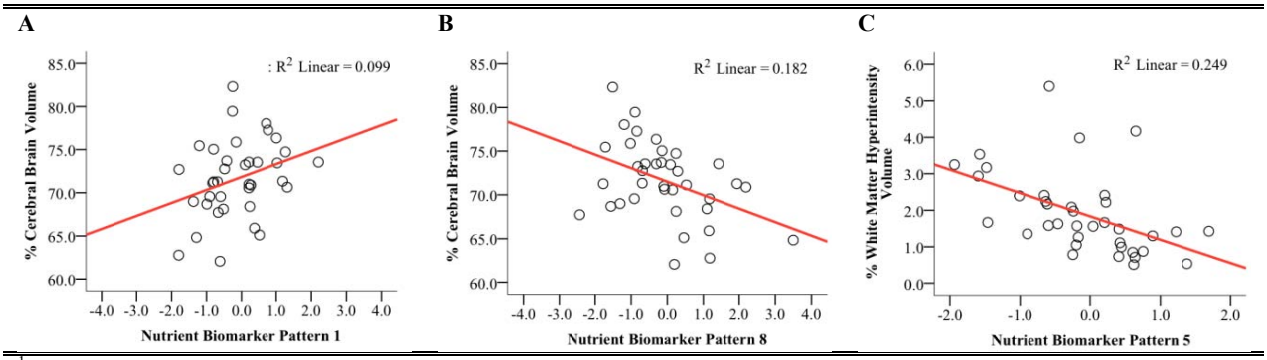
	Total Cerebral Brain Volume[2]			WMH Volume[3]		
	β	***SE***	***P***	***β***	***SE***	***P***
NBP1 (BCDE)	1.585	.721	.034*	-.068	.141	.631
NBP2 (saturated fat)	.643	.876	.470	.254	.171	.151
NBP3 (carotenoids)	-.192	.668	.776	-.063	.130	.636
NBP4 (cholesterol)	-.345	.738	.645	.095	.144	.516
NBP5 (omega 3)	-.529	.781	.504	-.331	.153	.040*
NBP6 (omega 6, retinol)	.834	.780	.295	.029	.152	.851
NBP7 (lutein, HDL)	.081	.818	.922	.253	.160	.125
NBP8 (trans fat)	-1.311	.572	.031*	.050	.112	.657

NBP, Nutrient biomarker pattern and the predominant nutrient biomarkers within each pattern
[1]All analyses are controlled for age, gender, and Mini Mental State Examination. Significant findings are color coded as favorable green and unfavorable orange
[2]Total cerebral brain volume expressed as a % of total intracranial volume to adjust for head size
[3]WMH, total white matter hyperintensity volume includes periventricular and subcortical deep signals and expressed as % of total cerebral brain volume to adjust for brain size

MRI scans were available in 42 subjects, all 85 years and older (mean age = 92.6, SD 3.8, range 85 – 101)(**table 2**). The mean age of this subset was higher that those without MRI by 9.4 years ($p < 0.0001$). Gender, education, MMSE, BMI, fasting duration, creatinine, comorbidity status, SES, drinking and smoking, and Hachinski ischemic score were not statistically different between those with and without MRI available (data not shown). The regression model adjusted for age, gender and global cognitive function (MMSE score) demonstrated three NBPs associated with MRI measures of brain aging: High NBP1 (BCDE pattern) associated with more total cerebral brain volume (TCBV) and this

remained significant after controlling for age, gender, MMSE score, and total intracranial volume (NBP1, $p = 0.037$). High NBP8 (trans fat pattern) associated with less TCBV and this significant relationship remained after the same covariate adjustment (NBP8, $p = 0.031$). High NBP5 (marine omega 3 pattern) was associated with less total white matter hyperintensity volume (WMH) and this remained significant after adjustment for age, gender, MMSE, and TCBV (NBP5, $p = 0.040$, **table 7**).

Figure 4. Direction and Magnitude of Variance Explained in Brain Volume by the Nutrient Biomarker Patterns[1]



[1]WMH includes periventricular and subcortical deep signals and is expressed as % of total cerebral brain volume to adjust for brain size; Cerebral brain volume expressed as a % of total intracranial volume to adjust for head size and excludes cerebellum and brain stem (supratentorial volume). All plots represent statistically significant linear associations ($p < 0.05$, **table 4**).

The vitamin B, C, D, and E pattern (NBP1) and the trans fat pattern (NBP8) explained 9.9% and 18.2% of the variance in total cerebral brain volume, respectively (**figure 4a and 4b**). The marine omega 3 pattern (NBP5) explained 24.9% of the variance in total WMH volume (**figure 4c**).

Association of Nutrient Biomarker Patterns with Rates of Decline in the Oregon Brain Aging Study

Table 8. Multivariate Linear Regression: Association of distinct NBPs with annual CDR-SOB change score

				95% CI for β	
	β	SE	*P*	Lower Bound	Upper Bound
(Constant)	1.946	2.199	.380	-2.450	6.343
NBP1 score	-.266	.098	.008	-.462	-.071
NBP2 score	-.183	.107	.093	-.398	.031
NBP3 score	-.010	.098	.917	-.205	.185
NBP4 score	-.132	.104	.209	-.341	.076
NBP5 score	-.066	.105	.529	-.275	.143
NBP6 score	.091	.098	.359	-.105	.287
NBP7 score	-.178	.094	.062	-.366	.009
NBP8 score	.362	.106	.001	.150	.575
Age	.003	.013	.822	-.023	.028
Gender	.132	.228	.564	-.324	.588
Education years	.029	.045	.530	-.062	.119
Mini Mental State Exam	-.085	.044	.057	-.172	.003
Multivitamin use	-.347	.224	.126	-.794	.100

OBAS, Oregon Brain Aging Study; NBP, Nutrient Biomarker Pattern; CI, confidence interval, CDR-SOB, Clinical Dementia Rating sum of box score. Dependent Variable: CDR-SOB, annual change

The longitudinal analysis identified 2 NBPs associated with rates of change in CDR-SOB score over a mean follow-up duration of 1.8 years: NBP1 (BCDE pattern) was associated with slower rates of decline and NBP8 (trans fat pattern) with more rapid rates of decline in univariate and multivariate analysis controlling for important potential confounders (age, gender, education years, MMSE score, and multivitamin use)(**table 8**).

CONCLUSIONS

This cross sectional study provides the initial description of how the nutrient biomarker patterns are structured using principal component analysis (PCA) in a population at risk for dementia. This novel application of existing methods identified three distinct nutrient biomarker patterns associated with cognitive and neuroimaging

outcomes: a plasma profile high in antioxidants (C and E), B vitamins and vitamin D (NBP1) associated with superior attention, executive, and visuospatial skills and less total brain atrophy. The marine omega 3 pattern (NBP5) was associated with better executive function and less white matter hyperintensity volume. High trans fat (NBP8) was associated with inferior cognitive performance in multiple domains and significantly greater brain atrophy.

Dietary patterns associated with cognitive decline or Alzheimer incidence have historically derived the patterns from food frequency questionnaire data (FFQ). Dietary intake can be indexed as "healthy" or "unhealthy" based on existing knowledge and examined in relation to disease risk.[96,117] Data driven cluster analysis can generate exclusive dietary patterns in a population *a posteriori*[98] and reduced rank regression combines existing knowledge and the data at hand to derive dietary patterns.[100] These relatively large FFQ studies have identified dietary patterns with higher dark and green leafy vegetables, cruciferous vegetables[100], fish[118], fruit[96,100] and lower intake of organ meats, red meat, high fat dairy[64], butter[100] and trans fat[66,68] as a promoter of cognitive function, but the results have lacked consistency. Our findings have reached some similar results using biological markers of diet. For instance, the plasma nutrient profiles that appeared favorable in our study (BCDE pattern, NBP1 and omega 3 pattern, NBP5) would be sensitive to frequent consumption of dark and green leafy vegetables, cruciferous vegetables, fruit, and fish. In addition, a plasma profile high in trans fat and retinol (NBP8) was associated with unfavorable brain outcomes and this would be expected in people frequently consuming bakery foods, margarine spreads[119], fried foods, red meat[119] and offal.[120] The consistency of our results with some FFQ studies is

encouraging and provides further impetus for utilizing biological markers of diet in studies of cognitive aging in older populations.

The neuroimaging results suggest heterogeneous mechanisms by which the two favorable nutrient biomarker patterns (NBP1 and NBP5) may be affecting cognitive function. Cognitive benefit gained by a plasma profile high in antioxidants C and E, B vitamins, and vitamin D may operate on the neurobiology that governs rate of total brain atrophy (e.g., Alzheimer's type pathology), whereas the effects of EPA and DHA (NBP5) may be mediated through more vascular mechanisms.[121,122] The vitamin B, C, D, and E combination may offer support for hippocampal neurogenesis[123], the reduction of β-secretase activity[124] and mute oxidative pathways that exacerbate β-amyloid pathology.[125] This nutrient combination may also reduce hyperhomocysteinemia induced neurotoxicity[126], improved blood brain barrier function[127] and the maintenance of high CSF to plasma ascorbic acid ratio important to rate of AD progression.[128]

An unfavorable nutrient biomarker pattern characterized by high trans fat (*trans* linolelaidic acid, 18:2 n-6*t,* NBP8) was a consistent finding in the current study. Linolelaidic acid is predominately found in bakery foods such as cookies, doughnuts, cakes, pastries and pies.[119] These foods are often prepared with hydrogenated vegetable oils to allow for a shelf life. Higher trans fatty acid intake has been associated with cardiovascular disease, systemic inflammation, and endothelial dysfunction, all of which may partially explain an association with cognitive function.[59,62] Very few studies have investigated dietary trans fat and risk for cognitive decline.[66] To our knowledge, studies of plasma trans fat and cognitive performance in elders have not been reported. However, some evidence supports the hypothesis that dietary trans fat may aggravate cognitive

function independently and jointly through interaction with other dietary factors. Another report suggests that trans fat displaces DHA in neuronal membranes, but does not affect the neuropathology of Alzheimer's disease in mice.[67] The inverse association we observed between plasma trans fat and total brain volume suggests neurologic consequences in man, but these findings need to be studies further before firm conclusions can be met.

In longitudinal analysis of CDR-sum of box score change over 1.8 years we identify two nutrient biomarker patterns as significant predictors of rates of change. NBP1, represented by high plasma concentrations of vitamins B, C, D, and E was associated with less change in CDR-SOB suggesting that this nutrient combination promotes a stabilization of cognitive and functional abilities over time. In contrast, NBP8, mostly represented by high plasma trans fat was associated with more change in CDR-SOB. This indicates that high plasma trans fat is a risk factor for cognitive and functional decline in subjects at risk for dementia.

The cross sectional analysis showed NBP1 and NBP8 consistently associated with better and worse brain outcomes, respectively. The prospective analysis establishes the temporal association between these distinct nutrient patterns and rates of cognitive decline. Biological plausibility of a relationship between nutrition and brain health is supported by several cellular, animal and human investigations highlighted in the introduction. Nutrition may affect the neurobiology of AD through various mechanisms that include oxidative stress, amyloid and tau metabolism, cerebral blood flow, neuronal energy metabolism and homocysteine mediated neurotoxicity, blood brain barrier function and other vascular mechanisms. The internal consistency, biologic plausibility

and temporal relationship between the BCDE pattern (NBP1) and trans fat pattern (NBP8) and cognitive decline argue in favor of a causal relationship between these nutrient pathways and neurodegenerative processes.

There are limitations of the current study. The PCA procedure may require investigator decisions with the data in hand. For example, using an eigenvalue of >1.0 as inclusion criteria for the number of nutrient patterns to carry forward to hypothesis testing is exploratory and may require more field specific criteria. Our nutrient biomarkers were selected *a priori* using existing knowledge of an association with neurodegeneration, but this may not reflect the ideal set of nutrients to study. Observational studies are susceptible to residual confounding, and the cross-sectional design utilized is not well suited for inferring any causal association since the temporal relationship between the nutrients and outcomes cannot be described and this is the first study of it's kind. However, our longitudinal findings establish temporality and argue against reverse causality. Our study population was restricted to a relatively healthy and well-educated cohort of white, non-Hispanic elders with minimal genetic risk for Alzheimer's disease. These attributes may or may not limit the generalizability of our results.

Future studies should apply these methods in separate and larger populations with greater ethnic diversity followed prospectively to predict cognitive and neuroimaging outcomes. Relevant gene-nutrient interactions underlying a relationship between nutrition and cognition should be investigated since *APOE4* carriers may benefit less from nutritional interventions.[58,97,129] The long-term reproducibility of the nutrients combinations should be evaluated for change in elder populations. Finally, these dietary

biomarkers should be evaluated at different clinical stages of cognitive function to decipher the most appropriate population for receiving such an intervention.

D. SUMMARY AND DUSCUSSION

Table 9. Summary of findings in OBAS

	Cross sectional study								Prospective study
	Cognitive function						Volumetric MRI		CDR-SOB
	Executive	Memory	Attention	Visuospatial	Language	Processing	TCBV	WMH	Cognitive decline
NBP1	Better		Better	Better			More		less
NBP2									
NBP3									
NBP4									
NBP5	Better							Less	
NBP6		Worse			Worse				
NBP7		Better							
NBP8		Worse	Worse		Worse	Worse	Less		More

This study indicates that principal component analysis can be applied to a diverse panel of dietary biomarkers to identify distinct nutrient biomarker patterns in a population. Our analysis identified 8 distinct nutrient biomarker patterns, 5 were associated with at least one cognitive domain, 3 associated with both cognitive and neuroimaging measures of brain aging, and 2 predicted rates of decline over a mean follow up period of 1.8 years. These are remarkable findings given the relatively small sample size (n=104) followed for such a short period. The results support the hypothesis that nutrient combinations may provide therapeutic value in promoting cognitive health. Future studies applying these methods will need to pay considerable attention to the collection and handling of biospecimens to minimize sources of error in the classification of dietary exposure using plasma biomarkers. Special attention in the following areas, including: 1) tube additives (i.e., heparin or edta anticoagulants used), 2) spinning and timing of plasma / serum separation, 3) cryostorage delay, 4) assay kit dilution procedures, and 5) fasting status of

the subject should be considered. Standardization of these methods across laboratories would be helpful in facilitating further studies of nutrition using dietary biomarkers. Finally, it is important to note that sources of information bias related to biological markers of diet maybe more manageable in older populations at risk for dementia than sources of error seen in less direct methods for dietary assessment (i.e., FFQ).

One explanation for the results could manifest from our focus on nutrients in combination rather than in isolation. We were interested in 30 dietary biomarkers that have been implicated in Alzheimer's disease. Many of these are highly interrelated making it difficult to interpret independent significance when each are tested in regression analysis simultaneously. For example, if we find a relationship between vitamin C and cognition, we are less certain of this relationship when we know that vitamin C is highly correlated with vitamin E or some other nutrient that may be the true culprit. Applying PCA to a set of highly interrelated nutrients essentially converts this statistical problem from a limitation into an advantage by assembling highly interrelated nutrients into their own distinct patterns or combinations. This is how PCA permits interaction in the model. When applied to biological markers of diet, the nutrient combinations are assembled on the basis of their circulating concentrations. Our approach may have identified a nutritional formula capable of operating on multiple pathways that modulate the biology of neurodegeneration.

The data suggest that specific cognitive phenotypes may be sensitive to distinct nutrient combinations in circulations (**table 9)**. Understanding which nutrient combinations may

have therapeutic potential in particular cognitive phenotypes would enable an individualized nutritional therapy aimed at promoting cognitive health. We found a NBP represented mainly by high plasma vitamins B, C, D, and E associated with better executive, attention and visuospatial skills, larger total cerebral brain volume and slower cognitive decline, but this pattern did not associate with the memory domain. In contrast, NBP8 mostly represented by high trans fat was associated with worse memory, attention, language, processing speed skills, more total brain atrophy and more rapid rates of cognitive decline. However, this NBP was not associated with executive function, visuospatial skills or cerebrovascular pathology for the brain. Further, NBP5 mostly represented by high plasma DHA and EPA, was associated with better executive function and less white matter hyperintensities of the brain, but not rates of cognitive decline nor total brain volume. These findings suggest that nutritional guidelines would ideally be tailored to the cognitive deficits presented by the subject.

Considering the short duration of follow up in this cohort study, the overall findings are extraordinary. The cognitive rate of change is difficult to predict in such a short time frame, particularly in subjects free of dementia at baseline. Most dietary studies of cognition include hundreds even thousands of subjects followed over several years. The magnitude of the association suggests that the effects of a distinct nutrient combination can be appreciated in a two-year period in a population at risk for dementia. More studies are needed in the area for a better understanding of consistency across populations with more diverse cognitive and neuroimaging outcomes. Gene-nutrient interactions may be important to consider therapeutically. For example, *APOE4* carriers may benefit less

from fish and DHA supplementation.[58,97,129] Studies are needed to describe the short and long-term reproducibility of nutrient biomarker profiles in elder populations at different levels of cognitive function / neurodegenerative disease. This would help decipher the appropriate design of an intervention's target population best suited for receiving the right nutrient mixture.

Biological markers of diet can be used as an objective means for classifying dietary intake in older populations.. Reducing exposure misclassification that exists equally in those developing and not developing cognitive deficits (non-differential) or reducing the misclassification that occurs unequally between the cases destined and free of cognitive deficit at follow up (non-differential) will minimize the probability of under or potentially overestimating the measures of association, respectively. This principle may explain some of the inconsistent findings in studies using dietary surveys as measures of nutritional exposure in relation to chronic disease in older populations. When measures classify nutrition very well, statistical power is gained without increasing sample size. This phenomenon is vital to the epidemiology of sporadic Alzheimer's disease, where long latency periods of development are the rule and disease outcome classification is also a concern. The sources of measurement error in biological markers of diet may be more manageable in older populations than dietary surveys and should be coupled with FFQ studies asking elders to recall their dietary intake.[109]

Principal component analysis (PCA) of fatty acids in serum and erythrocyte membranes has been conducted.[130,131] These study findings of eicosapentaenoic acid and

docosahexaenoic acid loading heavily together in one distinct pattern was confirmed in our older population using both relative and absolute concentrations in plasma. The mutual metabolism between EPA and DHA is one explanation for the collinearity between these two plasma fatty acids, and another is that they are found in similar foods. PCA constructs principal components (patterns/profiles/factors) on the basis of variable interrelatedness. The variability of this relatedness (collinearity) is explained by dietary intake and interactive metabolism, with duration of fasting as a tipping point factor in the algorithm [Y(plasma profile) = dietary intake + interactive metabolism + error]. When fasting duration is lengthened the plasma analytes are less of a reflection of dietary intake and more impacted by metabolic interaction. This effect modification is difficult to detect using PCA unless one compares both fasting and non-fasting biomarker profiles and their correlation structure after PCA. We provide evidence supporting the utilization of a data driven approach to the assessment of nutrient biomarkers as a successful avenue for studying nutrition in aging populations. Carotenoids (NBP3), total and LDL-cholesterol (NBP4), saturated fats (NBP2), and the omega 6 fatty acids (NBP6) loaded distinctly together and each of these patterns contain nutrients with known interactive metabolism. The observation that antioxidants (C and E), B vitamins, and vitamin D all load together is a new and intriguing observation. The impact of this nutrient combination on cognitive health should be studied further since this formula at higher concentrations in plasma appeared neuroprotective. The hypothesis that we can detect favorable and unfavorable nutrient/food combinations associated with clinically relevant brain outcomes is supported. Together, these results lay groundwork for the study of plasma nutrient profiles in brain aging research. The novelty stems from the use of

biological markers of diet and the application of PCA to these measures as an approach for rigorously studying synergistic nutrient combinations in relation to brain aging outcomes.

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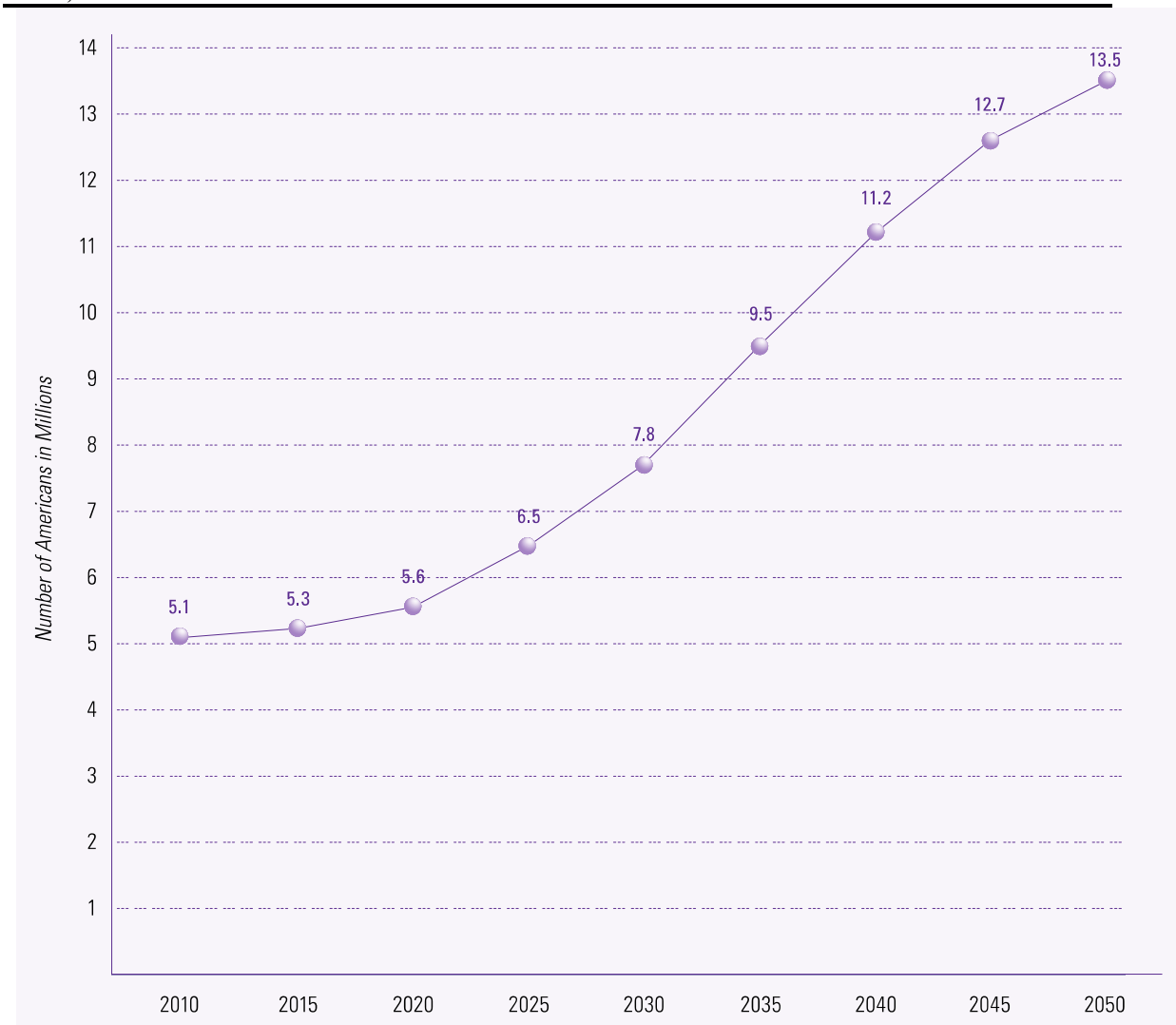
F. FIGURES

Figure 1. Current and Projected Prevalence of Alzheimer's disease in Americans Aged 65 and Older, 2010-2050[3]



Graph made available by the Alzheimer's Association using previously published data sources. Current prevalence and future projections for people < 65 years old is not available because that data does not currently exist.

Figure 2. Annual Rate of Change: Elderly and People of All Ages in Oregon [4]

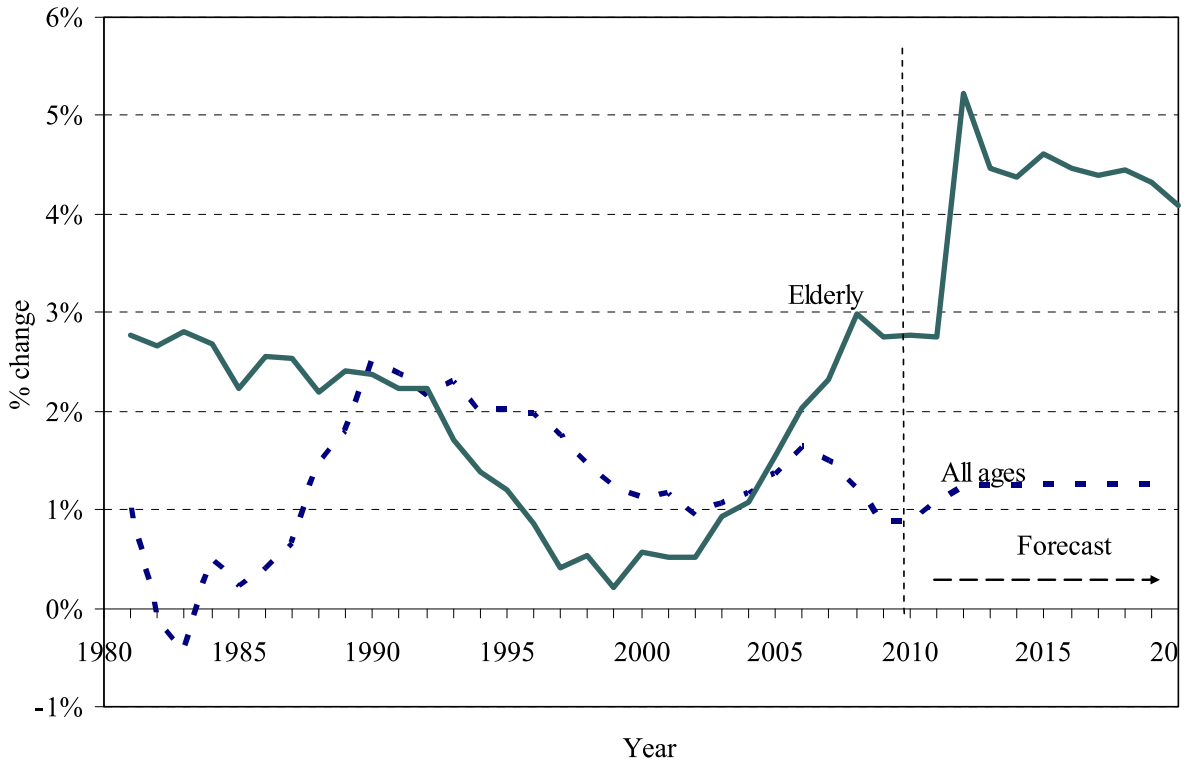


Figure 3. Estimated Cost of Care for Americans Age 65 and Older with Alzheimer's disease and Other Dementia, 2010-2050[3]

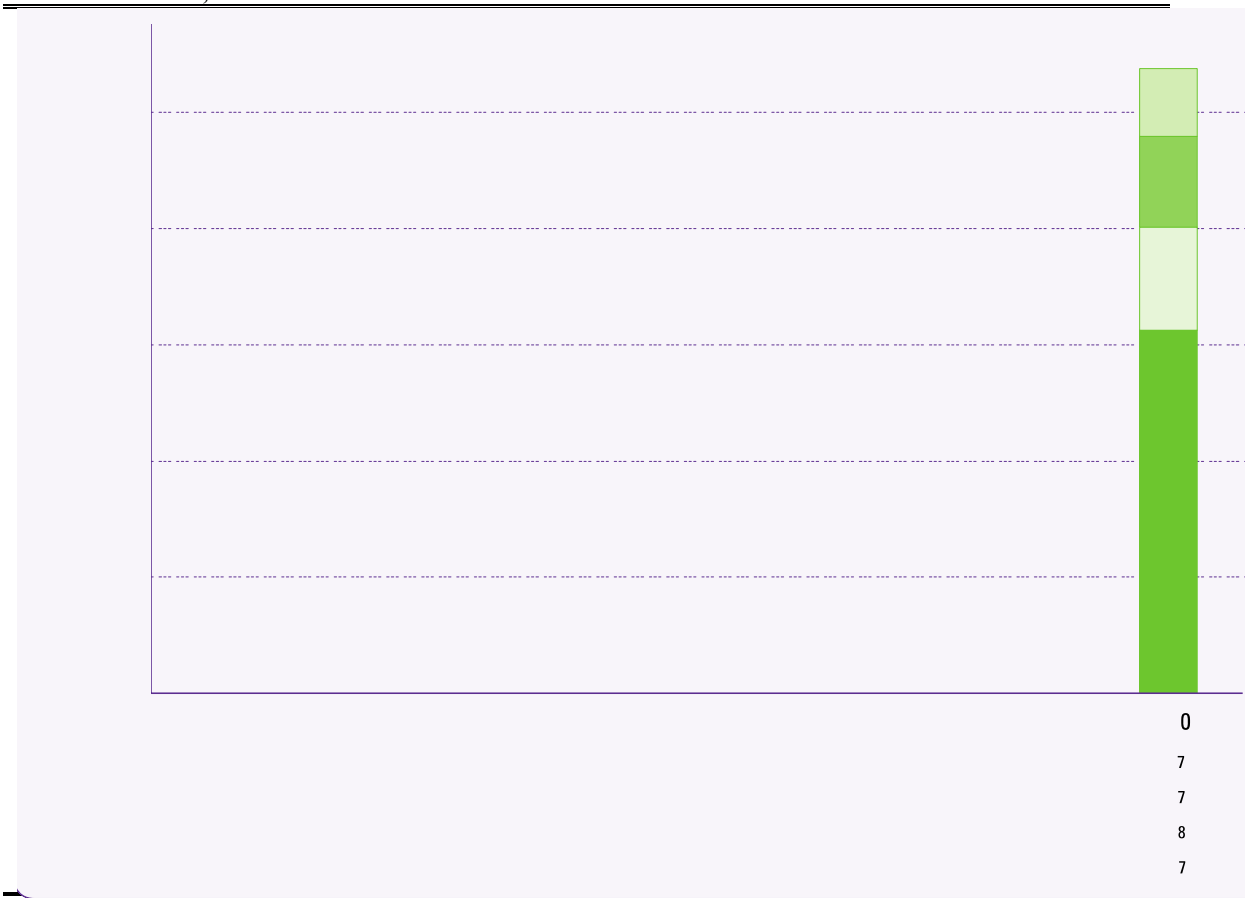
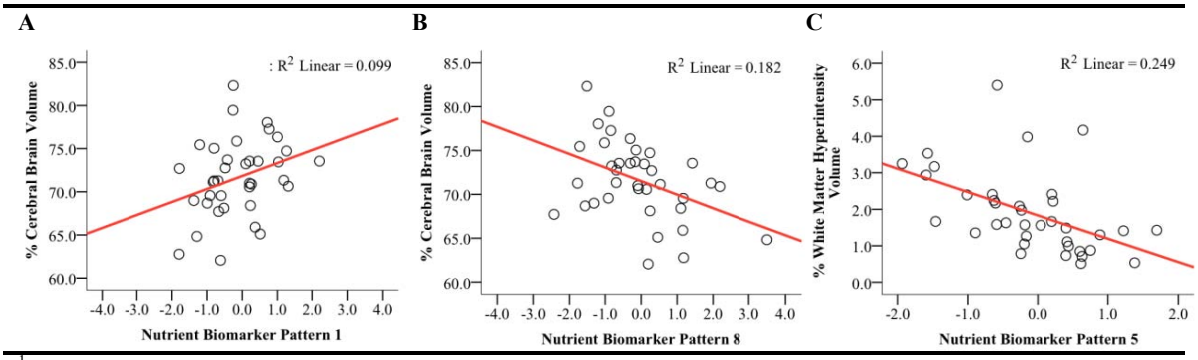


Figure 4. Direction and Magnitude of Variance Explained in Brain Volume by the Nutrient Biomarker Patterns[1]



[1]WMH includes periventricular and subcortical deep signals and is expressed as % of total cerebral brain volume to adjust for brain size; Cerebral brain volume expressed as a % of total intracranial volume to adjust for head size and excludes cerebellum and brain stem (supratentorial volume). All plots represent statistically significant linear associations ($p < 0.05$, **table 4**).

G. TABLES

Table 1. Epidemiological Studies of Serum Vitamin D, Cognitive Function and Risk for Dementia

Author	Design	Methods	Results
Wilkins et al [41]	Cross sectional, n=80 (40 with AD, 40 elder controls), men and women	Serum 25-OHD, cognition (Short Blessed Test, MMSE CDR-SOB) and mood (depression symptom inventory)	Lower 25-OHD assoc. with low mood and cognitive function (SBT and CDR-SOB)
Przybelski et al[42]	Cross sectional chart review, n=80, men and women	Serum 25-OHD MMSE	Positive correlation between 25-OHD and MMSE
Buell et al[43]	Cross sectional, n=318, >=65 yo men and women	Serum 25-OHD Dementia diagnosis MRI – WMH	Deficient 25-OHD assoc. OR 2.3 for all dementia Deficient 25-OHD assoc. with more WMH volume
Llewellyn et al[44]	Prospective, n=858, >=65 yo, men and women followed over a 6 year period	Serum 25-OHD MMSE, decline defined as 3 or more points Trails A and B, decline defined as worst 10% of the distribution of decline Random effects	Lower 25-OHD assoc. with cognitive decline
Slinin et al[45]	Prospective, n=1604, men followed on average 4.6 yrs.	Serum 25-OHD Logistic regression	No independent assoc. between 25-OHD with baseline 3MS or trails B, nor incident cognitive decline
Annweiler et al[46]	Cross sectional, n=752 women only >=75 yo	Serum 25-OHD Logistic regression Cognitive impairment = Pfeifer Short Portable Mental State Questionnaire < 8	25-OHD <10ng/mL assoc. with 1.9 OR of CI compared to >10 ng/mL
Llewellyn et al[47]	Cross sectional, n= 3325, >=65 yo men and women	Serum 25-OHD Logistic regression ORs Cognitive impairment = worst 10% of distribution	Increased OR of impairment per increment decrease in 25-OHD

Table 2. Epidemiological Studies Of Dietary Patterns, Cognitive Function, and Dementia

Author	Design	Methods	Results
Scarmeas et al[96]	Prospective of 2258 non demented elders with a mean follow up of 4 years	Dietary index of Mediterranean diet using FFQ; Nine categories: fruits vegetables, legumes, cereals, fish, meat, dairy, fat, alcohol (median cutoff 0 or 1)	Higher adherence to the MeDi was associated with lower risk for AD (HR, 0.91; 95% CI, 0.83-0.98; p=0.015). Compared with subjects in the lowest MeDi tertile, subjects in the middle MeDi tertile had a hazard ratio of 0.85 (95% CI, 0.63-1.16) and those at the highest tertile had a hazard ratio of 0.60 (95% CI, 0.42-0.87) for AD (p=0.007).
Barberger-Gateau et al[97]	Prospective study of 8,085 non-demented elders >=65 years old followed for mean of 4 years participating in the Three-City cohort study in Bordeaux, Dijon, and Montpellier (France)	Built a model considered brain healthy using FFQ based on existing knowledge and significant univariate relationships with the cognitive outcome.	Daily consumption of **fruits** and **vegetables** was associated with a decreased risk of all cause dementia (HR, 0.72, 95% CI 0.53, 0.97) -Weekly consumption of **fish** was associated with a reduced risk of AD (HR 0.65, 95% CI 0.43 to 0.994) and all cause dementia but **only among ApoE ε4 non-carriers** (HR 0.60, 95% CI 0.40 to 0.90) -Regular consumption of **omega-6 rich oils** not compensated by consumption of omega-3 rich oils or fish was associated with an increased risk of dementia (HR 2.12, 95% CI 1.30 to 3.46) **among ApoE ε4 non-carriers only**
Samieri et al[98]	Cross-sectional study of 1,724 elders living in Bordeaux, France from 2001 to 2002.	Cluster analysis using FFQ	Five dietary clusters were identified in each sex. A "healthy" cluster characterized by higher consumption of **fish** in men (n=157; 24.3%) and **fruits** and **vegetables** in women (n=267; 24.8%) had significantly lower mean number of errors to MMSE score (beta=-0.11; 95% CI, -0.22 to -0.004 in men; beta=-0.13; 95% CI, -0.22 to -0.04 in women).
Scarmeas et al[99]	Prospective study of 1393 cognitively intact elders followed on average of 4.5 years	Dietary index of Mediterranean diet using FFQ	-Compared with subjects in the lowest MeDi adherence tertile, subjects in the middle tertile had 17% less risk ([HR] = 0.83; 95% CI, 0.62-1.12; P = .24) of developing MCI and those in the highest tertile had 28% less risk (HR = 0.72; 95% CI, 0.52-1.00; P = .05) of developing MCI (trend HR = 0.85; 95% CI, 0.72-1.00; P for trend = .05). -Compared with subjects in the lowest MeDi adherence tertile, subjects in the middle tertile had 45% less risk (HR = 0.55; 95% CI, 0.34-0.90; P = .01) of developing AD . Those in the highest tertile had 48% less risk (HR = 0.52; 95% CI, 0.30-0.91; P = .02) of developing AD (trend HR = 0.71; 95% CI, 0.53-0.95; P for trend = .02).
Gu et al[100]	Prospective study of 2148 elders >=65 yo free of dementia followed on average 3.9 years	Reduced rank regression Using FFQ and informed by mediating biomarkers	A dietary pattern represented by higher intakes of **salad dressing**, **nuts, fish, tomatoes, poultry**, **cruciferous vegetables, fruits,** and **dark and green leafy vegetables** and a lower intake of **high-fat dairy products, red meat, organ meat, and butter** strongly associated with lower AD risk: compared with subjects in the lowest tertile of adherence to this DP, the AD hazard ratio (95% confidence interval) for subjects in the highest DP tertile was 0.62 (0.43-0.89) (*P* = .01)
Tangney et al[101]	Prospective study of 3790 (2280 blacks, 1510 whites) with mean follow up of 7.6 years	Dietary index comparing adherence to MeDi diet versus Healthy Eating Index (HEI)	Higher MeDi scores were associated with slower rates of cognitive decline (beta = +0.0014 per 1-point increase, SEE = 0.0004, P = 0.0004). No such associations were observed for HEI-2005 scores.

Table 3. Construction of the Cognitive Z-scores

Attention = $(Z_{\text{Digit Span Forward}} + Z_{\text{Digit Span Backwards}}) / 2$
Executive function = $(Z_{\text{Trail Making Test B}})$
Language = $(Z_{\text{Animal List}} + Z_{\text{Vegetable List}} + Z_{\text{Boston Naming Test}}) / 3$
Memory = $(Z_{\text{WMSR LM IA Immediate}} + Z_{\text{WMS LM IIA Delayed}} + Z_{\text{CERAD Word List Immediate}} + Z_{\text{CERAD Word List Delayed}}) / 4$
Processing speed = $(Z_{\text{Trail Making Test A}})$
Visuospatial function = $(Z_{\text{WMS-R Block Design}})$

Table 4. Demographic and Clinical Characteristics of the Oregon Brain Aging Study

		All N=104
Age, mean (SD), y		87 (10)
Female gender, No. (%)		64 (62)
Education, mean (SD), y		15 (3)
APOE4 carrier, No./total (%)		9/98 (9)
BMI, mean (SD), kg/m^2		25 (4)
BP, mean (SD), mm Hg		
Systolic		124 (17)
Diastolic		66 (10)
Creatinine, plasma, mean (SD), mg/dL		1.0 (0.3)
Hypercholesterolemia, No./total (%)		24/104 (23.1)
Smoking, No./total (%)		2/104 (2)
Drinking, No./total (%)		40/86 (39)
Diabetes, No./total (%)		5/104 (4.8)
Hypertension, No/total (%)		46/104 (44.2)
Depression, No./total (%)		22/104 (21.2)
Multivitamin use, No./total (%)		25/95 (26)
Fasting Duration, mean (SD), hours		3.3 (2.8)
Social Economic Status scale, mean (SD)		49 (10)
Hachinski ischemic scale score, mean (SD)		1.7 (2.0)
Neuropsychological Tests, mean (SD)		
Global	Mini Mental State Exam	27 (3)
	Clinical Dementia Rating	0.17 (.24)
Attention	Digit Span – Forward	6.4 (1.2)
	Digit Span - Backward	4.7 (1.2)
Executive function	Trail Making Test B	139 (79)
Language	Boston Naming Test	26 (4)
	Category fluency – Animals	17 (7)
	Category fluency – Vegetables	13 (6)
Memory	Logical Memory IA	15 (4)
	Logical Memory IIA	14 (5)
	Word-List Acquisition	19 (5)
	Word-list Recall	5.7 (2.7)
Processing speed	Trail Making Test A	44 (23)
Visual spatial	Block Design	23 (8)
Volumetric MRI, mean (SD), cm^3		***N = 42***
Total Intracranial Volume (TIV)		1164 (118)
Total Cerebral Brain Volume (TCBV)[1]		833 (89)
White Matter Hyperintensity Volume (WMH)		16 (10)
TCBV as % of TIV		72 (4)
WMH as % of TCBV		1.9 (1.2)

Abbreviations: *APOE4*, apolipoprotein E epsilon 4; MRI, Magnetic Resonance Imaging; Comorbidities require active treatment to qualify.[1]Total cerebral brain volume excludes brain stem and cerebellum

Table 5. Principal Component Analysis of Nutrient Biomarkers: Structure and Variance Explained[1]

Nutrient Biomarkers	Nutrient Biomarker Patterns							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Pyridoxal – 5 - phosphate (B6)	**.847**[§]				.210			
Thiamin (B1)	**.812**					-.208		-.269
Riboflavin (B2)	**.788**							.246
Folate (B9)	**.765**					.180		
Ascorbic acid (vitamin C)	**.649**		.265			.189	.264	-.180
α-Tocopherol (vitamin E)	**.631**	.277		.287				
Cobalamin (B12)	**.558**	-.208	.316	-.358		.279		
25-hydroxyvitamin D	**.555**	-.209			.343	.253		.215
α-Linolenic acid (18:3n-3)		**.866**						
Palmitic acid (16:0)		**.863**		.190	.219			
Triacylglycerol		**.841**					-.360	
Heptadecanoic acid (17:0)		**.750**			.280			
Linoleic acid (18:2n-6)		**.727**		.421		.188		
Trans - Elaidic acid (18:1n-9*t*)		**.646**	-.169	-.264	-.359			
α-Carotene			**.778**		.271		.272	
β-Cryptoxanthin			**.700**	.165			-.164	
β-Carotene	.403		**.691**		.267			
Lycopene			**.552**		-.365		.306	
LDL – Cholesterol				**.849**		.177		
Cholesterol		.246	.175	**.799**		.337	.240	
Eicosapentaenoic acid (20:5n-3)	.180		.160		**.808**	.204	.229	
Docosahexaenoic acid (22:6n-3)		.234	.185		**.804**			
Arachidonic acid (20:4n-6)				.231	.289	**.806**		
γ-Linolenic acid (18:3n-6)		.337		.192	.181	**.685**		
Retinol		.162			-.209	**.569**		.566
HDL - Cholesterol		-.239	.192	.256		.302	.669	
Lutein + Zeaxanthin			.476				.630	.222
Uric acid	-.200	.273			-.176		-.594	.261
Niacin (B3)	.208			.409			-.503	-.402
Trans - Linolelaidic acid (18:2n-6*t*)				.167		-.163		**.729**
% Variance explained by each NBP	**21**	**15**	**11**	**7**	**5.4**	**4.5**	**4.5**	**4.1**
Cumulative % of variance explained	**21**	**36**	**47**	**54**	**50**	**64**	**68**	**73**

[1]Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.
[§]Nutrient biomarker patterns are interpreted on the basis of the strongest nutrient loading coefficients within each pattern. For example, NBP1 is interpreted as a profile mostly represented by high circulating plasma B vitamins, vitamins C, E, and D (loading coefficients > 0.50). Each participant receives a summary score comprised of the linear combination of the predominant plasma nutrients within each pattern. Coefficients < 0.15 were excluded to simplify the table and bold face highlight the major nutrients within each pattern.

Table 6. Summary of Linear Regression Analysis: Association of Nutrient Biomarker Patterns with Cognitive Function[1]

	Cognitive Domain[2]											
	Executive		Memory		Attention		Visuospatial		Language		Process speed	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*
NBP1	-.213	.026*	.116	.164	.308	.002*	.214	.031*	.147	.077	-.165	.068
NBP2	.060	.536	.063	.463	.015	.880	-.002	.988	-.135	.118	-.105	.262
NBP3	.007	.945	-.043	.606	.181	.063	-.042	.664	.002	.983	-.075	.409
NBP4	-.120	.229	.025	.767	.042	.670	.047	.640	.016	.853	-.070	.459
NBP5	-.232	.037*	.061	.517	.017	.875	.025	.824	-.027	.774	-.125	.227
NBP6	-.137	.164	-.176	.042*	-.101	.311	-.045	.653	-.180	.039*	-.151	.107
NBP7	.067	.477	.230	.008*	.021	.828	.029	.767	.070	.407	-.051	.574
NBP8	.112	.239	-.200	.018*	-.260	.008*	-.083	.395	-.198	.018*	.208	.025*

NBP, nutrient biomarker pattern summary scores: NBP1, BCDE; NBP5, marine omega 3; NBP6, omega 6; NBP7, lutein and HDL-cholesterol; NBP8, trans fat.
[1]All analysis adjusted for age, gender and education. Significant findings are color coded: favorable green and unfavorable orange [2]Cognitive Z-scores

Table 7. Summary of Linear Regression Analysis: Association of NBP with MRI Measures of Brain Aging[1]

	Total Cerebral Brain Volume[2]			WMH Volume[3]		
	β	***SE***	***P***	***β***	***SE***	***P***
NBP1 (BCDE)	1.585	.721	034*	-.068	.141	631
NBP2 (saturated fat)	.643	.876	470	.254	.171	151
NBP3 (carotenoids)	-.192	.668	776	-.063	.130	636
NBP4 (cholesterol)	-.345	.738	645	.095	.144	516
NBP5 (omega 3)	-.529	.781	504	-.331	.153	040*
NBP6 (omega 6, retinol)	.834	.780	295	.029	.152	851
NBP7 (lutein, HDL)	.081	.818	922	.253	.160	125
NBP8 (trans fat)	-1.311	.572	031*	.050	.112	657

NBP, Nutrient biomarker pattern and the predominant nutrient biomarkers within each pattern
[1]All analyses are controlled for age, gender, and Mini Mental State Examination. Significant findings are color coded as favorable green and unfavorable orange
[2]Total cerebral brain volume expressed as a % of total intracranial volume to adjust for head size
[3]WMH, total white matter hyperintensity volume includes periventricular and subcortical deep signals and expressed as % of total cerebral brain volume to adjust for brain size

Table 8. Multivariate Linear Regression: Association of distinct NBPs with annual CDR-SOB change score

				95% CI for ***β***	
	β	**SE**	***P***	**Lower Bound**	**Upper Bound**
(Constant)	1.946	2.199	.380	-2.450	6.343
NBP1 score	-.266	.098	.008	-.462	-.071
NBP2 score	-.183	.107	.093	-.398	.031
NBP3 score	-.010	.098	.917	-.205	.185
NBP4 score	-.132	.104	.209	-.341	.076
NBP5 score	-.066	.105	.529	-.275	.143
NBP6 score	.091	.098	.359	-.105	.287
NBP7 score	-.178	.094	.062	-.366	.009
NBP8 score	.362	.106	.001*	.150	.575
Age	.003	.013	.822	-.023	.028
Gender	.132	.228	.564	-.324	.588
Education years	.029	.045	.530	-.062	.119
Mini Mental State Exam	-.085	.044	.057	-.172	.003
Multivitamin use	-.347	.224	.126	-.794	.100

OBAS, Oregon Brain Aging Study; NBP, Nutrient Biomarker Pattern; CI, confidence interval, CDR-SOB, Clinical Dementia Rating sum of box score. Dependent Variable: CDR-SOB, annual change

Table 9. Summary of findings in OBAS

	Cross sectional study								Prospective study
	Cognitive function						Volumetric MRI		CDR-SOB
	Executive	Memory	Attention	Visuospatial	Language	Processing	TCBV	WMH	Cognitive decline
NBP1	Better		Better	Better			More		less
NBP2									
NBP3									
NBP4									
NBP5	Better							Less	
NBP6		Worse			Worse				
NBP7		Better							
NBP8		Worse	Worse		Worse	Worse	Less		More