ω-3 FATTY ACIDS, HYPERTENSION AND RISK OF COGNITIVE DECLINE AMONG OLDER ADULTS: THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY

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ABSTRACT

MAY BAYDOUN: ω-3 Fatty Acids, Hypertension and Risk of cognitive decline among older adults: The Atherosclerosis Risk in Communities (ARIC) Study

(Under the direction of Jay S. Kaufman)

Cognitive impairment is a major health concern affecting loss of independence in basic daily activities in older age and thus special attention should be devoted to its prevention. Recent research indicates that ω-3 fatty acids, prominent in foods of marine origin, may also be important in preventing cognitive decline. So far, epidemiological evidence, although inconclusive, leans towards a protective effect of increased ω-3 fatty acid intake in the diet. Experimental animal studies suggest a plausible pathway by which hypertension and low dietary ω-3 fatty acid intake may interact in increasing the risk of cognitive decline.

The present study assessed the independent effect of low ω-3 fatty acid status on cognitive decline as well as the interaction of this risk factor with elevated blood pressure, as well as other factors associated with increased oxidative stress. The results of this study may have great public health and biomedical implications, particularly for prevention efforts among middle-aged adults. To this end, we conducted a secondary data analysis of the Atherosclerosis Risk in Communities (ARIC) study. This study initially recruited a probability sample of 15,792 men and women aged between 45 and 64 years from four distinct US communities, namely Jackson county (Mississippi), Forsyth county (NC), suburbs of Minneapolis (MN) and Washington county (MD).

Follow-up visits were conducted after baseline period (1987-89, or visit 1) on three occasions, separated by a three-year interval. Our analyses focused on men and women aged 50 years or more at visit 1. We assessed cognitive decline using three screening tools measured at visits 2 and 4. Exposure is assessed at visit 1 both in the diet (using a food frequency questionnaire) and in plasma (phospholipids and cholesteryl ester fractions). However, plasma fatty acids were measured only for the white population of MN at visit 1. Using empirical equations derived from animal feeding studies and several biomarkers, true fatty acid intake was predicted as well as measurement error which was corrected for in multivariate logistic models, using regression calibration and SIMEX methods.

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
AAMI	Age-Associated Memory Impairment
ABC	Health, Aging and Body Composition study
Aβ	Amyloid Beta peptides
Aβ42	Amyloid Beta 42
AC	Adenylate Cyclase
ACE	Acetyl Cholinesterase
ACL	Allen Cognitive Levels
ACTH	Adrenocorticotropic Hormone
AD	Alzheimer's Disease
ADAS	Alzheimer's Disease Assessment Scale
ADDTC	Alzheimer's Disease Diagnostic and Treatment Centers
ADL	Activities of Daily Living
AGECAT	Computerized diagnostic system for mental illness in the elderly
ApoE	Apolipoprotein E
APP	Amyloid Precursor Protein
ARCD	Age-Related Cognitive Decline
ARIC	Atherosclerosis Risk in Communities
ATH	Anti-Hypertensive Drug
AVLT	Audio-Verbal Learning Test
BCRS	Brief Cognitive Rating Scale
BDS	Blessed Dementia Scale
BEHAV	Behavioral factors
BLSA	Baltimore Longitudinal Study of Aging
BMI	Body Mass Index
BP	Blood Pressure
BSF	Benign Senescent Forgetfulness
C20	20-carbon
C22	22-carbon
CA	California
CAA	Cerebral Amyloid Angiopathy
c-AMP	Cyclic Adenosine Mono-Phosphate
CASI	Cognitive Abilities Screening Instrument
Ca-ATPase	Calcium Adenosine Triphosphatase
Ca^{2+}-CM-PKs	Calcium calmodulin protein kinases
CAMCOG	Cambridge Cognitive Examination
CAMDEX	Cambridge Examination for Mental Disorders of the Elderly
CCF	Coordinating Center Field
CCSE	Cognitive Capacity Screening Examination
CDR	Clinical Dementia Rating
CERAD	Consortium to Establish a Registry for Alzheimer's Disease

CERAD-NAB	Consortium to Establish a Registry for Alzheimer's Disease - Neuropsychological Assessment Battery
CES-D	Center of Epidemiologic Studies-Depression Scale
CHD	Coronary Heart Disease
CI	Confidence Interval
CIND	Cognitive Impairment, No Dementia
CFA	Confirmatory Factor Analysis
CFEI	Consumer and Food Economics Institute
CFS	Cognitive Function Scanner
CO-MB	Co-Morbid Conditions
COPD	Chronic Obstructive Pulmonary Disease
CRH	Corticotropin Releasing Hormone
CSF	Cerebro-Spinal Fluid
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DGLA	Dihomogammalinolenic Acid
DHA	Docosahexaenoic Acid
DLB	Dementia with Lewy bodies
DPA	Docosapentaenoic Acid
DSST	Digit-Symbol Substitution Test
DSMIII	Diagnostic and Statistical Manual – 3rd edition
DSMIII-R	Diagnostic and Statistical Manual – 3rd edition, revised
DSM-IV	Diagnostic and Statistical Manual – 4th edition
DWRT	Delayed Word Recal Test
EBMT	East Boston Memory Test
ECA	Epidemiologic Catchment Area Study
EDTA	Ethylene Diamine Tetra-Acetic acid
EFA	Exploratory Factor Analysis
ELISA	Enzyme-linked Immunosorbant Assay
EPA	Eicospentaeoic Acid
EPESE	Established Population for Epidemiological Study
EXM	Exam
EXP	Exposed
FA	Factor Analysis
FA	Fatty Acid
FDG-PET	[^{18}F]-fluoro-2-deoxyglucose positron emission tomography
FEV_1	Forced Expiratory Volume in 1 second
FFQ	Food Frequency Questionnaire
FFAP WCOT	Type of glass capillary column
f-MRI	Functional Magnetic Resonacne Imaging
FVC	Forced Vital Capacity
GBCF	Global Baseline Cognitive Functioning
GCD	Global Cognitive Decline
GDS	Global Deterioration Scale
GFA	Glial Fibrillary Acidic protein
GLA	Gamma-Linoleic Acid

Glm	General Linear Models
GMS	Geriatric Mental Schedule
HBP	High Blood Pressure
HDL-C	High Density Lipoprotein Cholesterol
Hg.	Mercury
HRT	Hormone Replacement Therapy
HUFA	Highly Unsaturated Fatty Acids
IADL	Instrumental Activities of Daily Living
ICD-10	International Classification of Diseases – 10^{th} edition
IFG	Impaired Fasting Glucose
IRR	Incidence Rate Ratio
LA	Linoleic Acid
LASA	Longitudinal Aging Study Amsterdam
LC	Long Chain
LCD	Limited Cognitive Disturbance
LDL-C	Low Density Lipoprotein Cholesterol
LNA	Linolenic Acid
MCD	Mild Cognitive Disorder
MCI	Mild Cognitive Impairment
MD	Minimal Dementia
MD	Mixed Dementia
MEDIC	Medications
MIP	Maximal Inspiratory Pressure
MMMS or 3MS	Modified Mini-Mental State Exam
MMSE	Mini-Mental State Exam
MN	Minneapolis
MND	Mild Neurocognitive Disorder
MONICA	Multinational monitoring of trends and determinants in cardiovascular disease
MoVIES	Monongahela Valley Independent Elderly Survey
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MUFA	Mono-Unsaturated Fatty Acids
MV	Manifest Variables
n-3	Omega-3
n-6	Omega-6
n/a	Not Applicable
NBP	Normal Blood Pressure
NCEP	National Cholesterol Education Program
NCSE	Neurobehavioral Cognitive Status
NFT	Neurofibriallary Tangles
n/h	No History
NHS	Nurses' Health Study
NIA	National Institute on Aging
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke -- the Alzheimer's Disease and Related Disorders Association

NINCDS-AIREN	National Institute of Neurological and Communicative Disorders and Stroke--Association Internationale pour la Recherche et l'Enseignement en Neurosciences.
NNFI	Non-Normed Fit Index
n/s	Not Specified
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NSHD	National Survery of Health and Development
NTP	Neural Threat Protein
NUTR	Nutritional factors
OA	Oleic Acid
OD	Other Dementias
OLS	Ordinary Least Square
OR	Odds Ratio
PA	Pennsylvania
PAQUID	Personnes agées, quid?
PCA	Principal Components Analysis
PD-D	Parkinson's disease with dementia
PD-Plus	Parkinson Plus syndrome
PET	Positron Emission Tomography
PGI	Prostaglandins
PHF	Paired Helical Filaments
PKA	Protein Kinase A
PKC	Protein Kinase C
PLA_2	Phospholipase A_2
PLC	Phospholipase C
PLD	Phospolipase D
Pr	Probability
PSP	Progressive Supranuclear Palsy
PUFA	Poly-Unsaturated Fatty Acids
QD	Questionable Dementia
RCAL	Regression Calibration
RCI	Reliable Change Index
RCT	Randomized Controlled Trial
RMSEA	Root Mean Square Error of Approximation
ROS	Reactive Oxygen Species
RR	Relative Risk
RSS	Residual Sum of Squares
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Structural Equations Modelling
SIMEX	Simulation Extrapolation
SMC	Squared Multiple Correlations
SOCI-D	Socio-Demographic
SP	Space
SPECT	Single photon emission computed tomography
SPMSQ	Short Portable Mental Status Questionnaire

TAG	Tri-Acyl Glycerol
TCA	Tricyclic Anti-depressants
SIMEX	Simulation Extrapolation
TIA	Transient Ischemic Attack
TICS	Telephone Interview for Cognitive Status
UN	United Nations
UNEXP	Unexposed
UFA	Unsaturated Fatty Acids
VaD	Vascular Dementia
vWF	Von Willebrand factor
WAIS	Wechsler Adult Intelligence Scale
WAIS-R	Wechsler Adult Intelligence Scale-Revised
ω-3	Omega-3
ω-6	Omega-6
WFT	Word Fluency Test
5-HT_1	5-Hypdroxy-tryptamine receptor 1
5-HT_2	5-Hypdroxy-tryptamine receptor 2
3H	*ω*-3 Highly Unsaturated Fatty acids (HUFAs) Mainly DHA+EPA
3P	*ω*-3 Polyunsaturated Fatty Acids (PUFAs) Mainly LNA

6H	*ω*-6 Highly Unsaturated Fatty acids (HUFAs) Mainly AA
6P	*ω*-6 Polyunsaturated Fatty acids (PUFAs) Mainly LA
3	Total ω-3 fatty acids
6	Total ω-6 fatty acids
3H/6H	Ratio of 3H over 6H
3P/6P	Ratio of 3P over 6P
3/6	Ratio of 3 over 6

Chapter 1

INTRODUCTION

The proportion of the elderly segment of the US population (65 and over) based on recent UN estimates was 12.3% in 2000 and is projected to increase rapidly in the coming few decades to reach 20% in 2050 (1). Population aging in a country like the US carries great social, economic and public health implications. Some of these implications include larger expenditures on pensions and health care, need for social security reforms and shrinking of the workforce and hence shortage of active persons that are able to support dependent older adults. In addition, with the growing number of elderly persons, health care has to expand so as to encompass the increasing demands for geriatrics services that are more costly to provide. The health implications should not be looked at from the sole perspective of cause of death structure. In developed countries like the United States, the problem of population aging lead to recognizing the importance of functional status in later life. Consequently, rather than focusing on extending life expectancy or reducing the risk of common chronic diseases, the national health objectives for the year 2000 targeted increasing healthy years lived and reducing limitations in activities of daily living or disability among the older segment of the population (2) . Disability may be defined as difficulty doing activities in any domain of life (from hygiene to hobbies, errands to sleep) due to a health or physical problem. The concept of disability is often operationalized in terms of limitations in Activities of Daily Living (ADL), mobility and Instrumental Activities of Daily Living (IADL).

Risk factors for incidence of disability or functional status decline can be grouped under health-related and behavioral, demographic and socio-economic factors (3). A review of 78 longitudinal studies showed that among the health-related and behavioral risk factors for functional status decline, those that had the strongest evidence included: cognitive impairment, elevated or low body mass index, disease burden or co-morbidity, reduced level of physical activity, smoking, poor self-rated health and visual impairment (4). According to a study by Moritz and colleagues (5), persistent incident ADL limitations occurred more frequently in persons who scored four or more errors on the Short Portable Mental Status Questionnaire (SPMSQ) for cognitive impairment as compared to those who scored zero. The observed odds ratios ranged between 2.72 for males and 2.60 for females after adjusting for age, race, history of chronic health conditions and incident health conditions. Another prospective cohort study was conducted on around 5,700 men and women whose mean age was 77 years and were followed up between 1993 and 1995. The main purpose of that study was to determine the relative contributions of cognitive impairment and depressive symptoms on decline in activities of daily living (ADL) function over a period of 2 years among older adults. Using a modified version of the Mini-Mental State Exam (MMSE) to measure cognitive impairment and the Center of Epidemiologic Studies-Depression scale (CES-D) to measure depression and a limitation in at least one of the six ADLs as the outcome, results indicated that cognitive impairment and depression are independent risk factors of functional status decline among those who were free of ADL limitations at baseline, but that only cognitive impairment affected the outcome significantly among those who were ADL dependent at onset of follow-up. These findings suggested that cognitive impairment although interrelated with depression in old age, can affect functional dependence in an independent way and hence resulting in a higher caregiver burden and elevated health care costs (6).

Similar findings were reported by others (7, 8). Therefore there is reason to believe that cognitive impairment is a major health concern that affects dependence in basic daily activities in older age and thus special attention should be devoted to preventing its occurrence.

C h a p t e r 2

REVIEW OF THE LITERATURE

A. Conceptual Framework

A.1. Fatty Acids and Brain Function

Linoleic(LA ~ 18:2n-6)[1] and α-linolenic (LNA ~ 18:3n-3) are two types of fatty acids that are essential for all members of the animal kingdom. These fatty acids and their respective derivatives are also commonly referred to as ω-6 and ω-3 (or n-6 and n-3). Their essentiality lies in the fact that they cannot be synthesized *de novo* within the human or animal organism. The main reasons are that the lack of Δ^{12} desaturase enzyme in mammals prevents conversion of oleic acid (OA ~ 18:1n-9) into linoleic acid and the absence of the Δ^{15} desaturase precludes interconversion of ω -3 and ω -6 fatty acids in man (9). In addition, they are essential because they are responsible for several biochemical and biophysical functions, which include structural integrity and fluidity of membranes, enzyme activities, lipid-protein interactions and as precursors for eicosanoids such as prostaglandins, leukotrienes and thromboxanes (10). In the past, ω -3 fatty acids were only classified as essential because of their ability to alleviate deficiency symptoms which include dermatitis, growth retardation and reproductive failure.

[1] The terms within parentheses refer to the acronym of the fatty acid followed by its chemical structure separated by ~. The chemical structure is as follows: "Total number of carbon atoms" : "# of double bonds" – "carbon number with first double bond starting from the methyl end".

However, ω -3 fatty acids do have other important neurological functions, which explain their high concentrations in neural and retinal tissues (11-13). Some of the longer chain fatty acids that are synthesized from α-linolenic acid include Eicosapentanoic acid (EPA ~ 20:5 ω -3), which through further elongation, desaturation and β-oxidation produces Docosahexaenoic acid (DHA ~ 22:6 ω -3). On the other hand, products of linoleic acid which are also termed long-chain ω-6 fatty acids include gamma-linoleic (GLA ~ 18:3 ω -6), dihomogammalinolenic acid (DGLA ~ 20:3 ω -6) and Arachidonic acid (AA ~ 20:4 ω -6). In both metabolic processes, the first step involves the activity of a Δ^6 desaturase enzyme, followed by an elongase, a Δ^5 desaturase, another elongase and finally a Δ^4 desaturase. The recommendations for dietary intake of Essential Fatty Acids (EFAs) have been set at 3-6% of total fat. Among these, linolenic acid should comprise around 1-2% for optimal production of EPA and DHA which have crucial roles for neuronal and visual tissue growth (14). While LNA can be found in most green leafy vegetables and some fruits as well as in some plant oils and nuts (e.g. rapeseed oil, butternut oil, flaxseed oil and English walnut), all other types of ω-3 fatty acids are found almost exclusively in fatty fish (e.g. salmon, tuna and mackerel) and fish oils (10). In contrast, AA is prominent in eggs and meat, while LA is found in most commonly used cooking oils (e.g. sunflower, safflower, corn oils).

Of all organs in the human body (excluding adipose tissue), the nervous system has the highest lipid content. The dry weight of an adult brain is 50% to 60% lipid, and 35% of the lipid content is accounted for by polyunsaturated fatty acids (PUFAs) (15). Tinoco and colleagues (16) reported that the phospholipids fraction of the brain contained very little Linoleic Acid (LA). In addition, Arachidonic Acid (AA) was found to be an important component of brain phospholopids although the most prominent polyunsaturated fatty acid (PUFA) was Docosahexaennoic acid (DHA) (17, 18).

A review of scientific articles and biochemistry textbooks (19) suggested that the fatty acid composition of neuronal cell membrane phospholipids reflects their intake in the diet. Fish oils, which contain high levels of C20 and C22 polyunsaturated fatty acids (PUFAs), exert the most profound influence on brain PUFA concentrations. Animals that were fed diets deficient in ω-3 fatty acids displayed considerably less DHA in the cerebral cortex as compared to those fed a balanced diet, and this deficit is usually compensated by an increase in brain DPA in its ω-6 fatty acid form (DPA ~ 22:5 ω -6), the elongated and unsaturated metabolite of AA (10). In contrast, astrocytes from the cortex of hamsters that were cultured with a DHA-rich medium had an improved physiological status, grew better, and retained their phenotype as compared to those cultured with AA. These astrocytes, in addition to uptaking DHA from the medium to alter the ω -6/ ω -3 PUFA ratio, had an increased level of EPA through active retro-conversion of DHA to EPA (20).

The importance of ω -3 PUFAs was reported by Yamamato and colleagues (21) who found that rats fed LNA had a longer mean survival time and increased learning ability in senescence. It seems unlikely that these effects were due to LNA itself, as very little concentrations are retained in brain membranes, and to date, no biological functions have been reported for this fatty acid, except as that of a precursor to longer chain ω -3 PUFAs. Several behavioral aspects of brain function have also been shown to be affected by dietary FAs. For example, rats fed a safflower oil diet, which has a ω -6: ω -3 FA ratio greater than 75, through two generations exhibited significantly lower phospholipids levels of DHA (around 90% less than a control group fed soybean oil diet which has a ratio of ω -6: ω -3 of 7).

Presumably, the high levels of LA in safflower oil down-regulated ω -3 essential Fatty Acid (EFA) de-saturation, resulting in the loss of membrane ω -3 PUFAs. Reflexes and motor abilities developed normally in both dietary groups, but deficient rats did exhibit fewer exploratory behaviors. They also performed more poorly in maze-learning tasks (22). Yehuda and Carasso (23) found that treatment of rats with preparations having a ratio of LNA:LA ranging between 1:3.5 and 1:5 were effective in improving the rate of learning, retention of old learning, pain thresholds and prevention of the d-amphetamine-induced hypothermic response to reduced ambient temperature. Similar findings were reported by other more recent studies (24-26). It has also been observed that rats fed on a diet low in ω -3 fats perform poorly in brightness discrimination (27) and shock avoidance tasks (28).

A wealth of studies has highlighted the important biological roles played by lipids. The degree of a fatty acid's de-saturation determines its 3-dimensional structure and thus its biophysical properties. DHA plays an essential role in brain membranes, the most notable of which are maintenance of "membrane fluidity" which may modulate a number of lipid-protein interactions, including certain enzyme activities. The ratio between ω-3 and ω-6 polyunsaturated fatty acids (PUFAs), in particular, influences various aspects of serotoninergic and catecholaminergic neurotransmission, as shown by studies in animal models. The exact mechanism by which lipids affect enzymes or transporters is still unclear. However, it has been shown that by increasing the density of neurotransmitter receptors for acetylcholine and dopamine, dietary ω -3 PUFA can improve learning and memory processes (29).

Within brain membranes, PUFAs have been shown to influence a biological pathway by increasing the activity of two enzymes namely Adenylate Cyclase (AC) and Protein Kinase A (PKA) which drive the c-AMP messenger system used by serotonin (5-HT_1), noradrenaline and adrenaline (α_2 and β-adrenergic), as well as dopamine (D_1 and D_2) receptors (30-32). In addition, PUFAs can affect 5-HT_2 and α_1 adrenergic transmission by exerting their effect on phospholipase C (PLC) and protein kinase C (PKC) (33, 34).

Two other enzymes, phospholipases D and A_2 (PLD and PLA_2) can play an important role in neurotransmission. PLA_2 has been shown to be activated by several receptors of neurotransmitters including dopamine (D_2), serotonin (5-HT_2), glutamate and muscarinic acetylcholine receptors (19). Moreover, PLA_2 can release AA, DGLA, and EPA from the sn-2 position of membrane phospholipids, but with markedly differing consequences. In fact, DGLA, AA and EPA can be transformed into prostaglandins (PGl) of the 1-, 2-, and 3-class, respectively. While the PGl_2 is highly pro-inflammatory, PGl_3 is anti-inflammatory and PGl_1 was shown to have intermediate properties. It has been hypothesized that a highly reactive PLA_2 is found in various psychiatric disorders (35). This high reactivity, when coupled with an elevated concentration of ω-6 fatty acids in brain membranes would lead to aggravated inflammatory conditions and development of neuronal dysfunction manifesting itself in psychiatric disorders. This condition can potentially be countered by the presence of sufficient ω-3 fatty acids in brain phospholipids. However, more data is needed to make definite conclusions about a potential for treatment. PUFAs can also modulate ion channels (mainly calcium and sodium channels).

Their influence on enzymes responsible for the release of neurotransmitters from synaptic vesicles namely Ca^{2+}-CM-PKs have been noted (19). In addition, Kearns and Haag (36) have noted that DHA and EPA can inhibit the enzyme Ca-ATPase in neuronal membranes of rat cerebral cortex, which is responsible for maintaining a thousand-fold concentration gradient between intra-cellular and extracellular calcium levels. They also found that the synaptosomal $Na^{+}K^{+}$ ATPase was inhibited by a high concentration of both ω-3 fatty acids. These results suggested a mechanism explaining the dampening effect of ω-3 fatty acids on neuronal activity. Although these sets of evidence of an effect of essential fatty acids on brain biochemistry and cognitive functions seem fragmentary, Yehuda and colleagues (37) proposed a unifying model which involved the hypothalamic-pituitary-adrenal axis.

According to this model, essential fatty acids are involved in neurotransmitters in the brain and hypothalamus, in releasing CRH and ACTH, and in the production of cortisol from cholesterol by P450. The enzyme P450 is involved in dopamine (the first molecule in the axis) and cortisol production (which is the end product of the axis) and thereby accounts for the ability of cortisol in the blood to affect brain function. However, for this axis to actually produce the end product "cortisol", cholesterol must be bioavailable in the brain a state that is highly dependent on membrane fluidity and hence on the ratio of ω-3 to ω-6 fatty acids.

The aging process can carry with it several biological changes. In spite of conflicting results, it was generally found that Δ^6 desaturase activities in the brain and in the liver which are crucial for the synthesis of long chain PUFAs from LA and LNA decline with aging. Interestingly, increased dietary intake of essential fatty acids can influence age-related changes in desaturase activity (38-40).

Consequently, even though high consumption of LA and LNA would have a reduced impact on the synthesis of longer-chained PUFAs, the direct intake of C20 and 22 PUFAs would avoid this limiting factor. While changes in PUFA synthesis with aging are important to consider, due attention should also be paid to the role of oxidative stress (OS), which refers to the cytopathologic consequences of a mismatch between the production of reactive oxygen species (ROS) and the cell's ability to defend itself against them. Many studies found evidence of an increase in ROS with age and their deleterious effects on lipids, especially PUFAs. The increase in lipid peroxidation in turn affects the oxidation of structurally important proteins disrupting transmembrane ion movements and cellular metabolic processes (41, 42), the most notable one of which is brain synaptic function. Although deprivation from ω-3 fatty acids leads to decline in DHA in the brain of animals, with aging, these animals will no longer be able forfeit their brain stores and instead DHA level will drop from other organs.

These findings suggest the necessity to preserve brain DHA levels, though at the expense of other organs (43). Unfortunately, DHA, with its high degree of unsaturation, may be a vulnerable target to the damaging effects of lipid peroxidation (10).

A.2. Hypertension, Fatty Acids and Brain Function

Hypertension is known to produce end-organ damage. Consequently, it is reasonable to postulate that neuropsychological deficits resulting from brain damage may occur as a consequence of high blood pressure levels. Psychomotor speed, visual constructive ability, learning, memory, and executive ability seem to be particularly vulnerable to increases in blood pressure (44-46). Possible pathways linking high blood pressure to poor cognitive functioning included metabolic imbalance, clinically silent stroke, atherogenesis, altered distribution of cerebral blood flow, as well as demyelination or microinfarction in the cerebral white matter (47).

The question remains: Is there a biological interaction between hypertension and intake of ω-3 fatty acids? Animal model studies demonstrated that hypertensive rats tend to have lower brain mono-unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) than normotensive rats (29). Since hypertension is a vascular type of pathology, altered fatty acid transport through the blood-brain barrier (BBB) may be one factor for the hypertension-related difference. While endothelial cells of the BBB were shown to selectively transport PUFAs and their precursors, astrocytes actively participate in their elongation to produce ω-3 and ω-6 fatty acids in the brain (48, 49). Although no prior studies have specifically looked at the effect of hypertension on fatty acid transport across the BBB, analogy can be made with other biochemical substances such as tryptophan and glutamic acid (50). In addition, perivascular astrocytic metabolism was also found to be compromised in hypertensive rats (51).

In short, either pressure-induced endothelial dysfunction at the BBB or exhausted astrocytic metabolism could lead to reduced MUFA and PUFA contents in the brains of hypertensive rats. An alternative explanation which was adopted by other researchers was that oxidative stress which accompanies high blood pressure will lead to increased peroxidation of unsaturated fatty acids and a reduction in their concentration in the brain. Consequently, there is a biologically plausible mechanism of interaction between elevated blood pressure and intake of ω-3 fatty acids, both of which affect fatty acid content and composition in the brain and therefore determine cognitive functioning.

In sum, the biological mechanism by which long-chain ω-3 fatty acids may affect brain functions has been studied extensively using animal models as well as tissue cultures. Although many of these studies did not explicitly investigate our outcome of interest (i.e. cognitive functioning), they did point to the important role played by ω-3 fatty acids in determining the biophysical characteristics of brain membranes which are crucial for many enzymatic actions. In addition, the biochemical composition of phospholipids is also influenced by this class of fatty acids and in turn affects the extent of inflammatory reactions in the brain that may explain many psychiatric disorders including cognitive impairment. Furthermore, oxidative stress which increases with aging renders these essential fatty acids extremely vulnerable to depletion especially that one of the enzymes that is responsible for their synthesis also becomes less active with age. Consequently, their direct intake from diet becomes crucial in preserving a balanced amount of long-chain ω-3 fatty acids in the brain. Moreover, based on the evidence presented above, there is reason to believe that reduced intake of ω-3 fatty acids and hypertensive status may interact to increase the risk of cognitive decline. Therefore, there may be a combined benefit of reducing blood pressure and supplementing diet with ω-3 fatty acids.

B. Historical Background

B.1. Cognitive Impairment and Prognostic Outcomes

Cognitive function refers to those mental processes that are crucial for the conduct of the activities of daily living. Such mental processes include attention, short-term (working) memory, long-term memory, reasoning, the coordination of movement and the planning of tasks. All of these processes vary in how well they are operating: sometimes our attention is poor; sometimes our memory seems excellent, sometimes we plan our activities badly, etc.

Therefore, the efficiency with which these processes are operating clearly has direct relationship to our ability to conduct everyday activities and thus, ultimately influences important aspects of the quality of life. Over the past three decades, psychologists have sought to improve the quality of cognitive function assessment in drug development. There is now an emerging discipline of Human Cognitive Psychopharmacology, the fundamental tenets of which are:

(1) There are major areas of cognitive function (e.g. attention, working memory), which underpin everyday behaviour. In fact, impairment can be attributed to separate domains of cognition. For example, the diagnosis of *dementia* requires the presence of impairment in memory and at least one other domain. Although it is hardly possible to divide cognition into mutually exclusive areas, the following domains can be regarded as relatively independent (52): **(A) Memory**: There are several types: *a. Episodic memory*: anterograde amnesia (severe loss of memory of recent events); retrograde amnesia (Forgetfulness for events predating the onset of impairment);

b. Semantic memory: knowledge of concepts or facts. **(B) Language**: They are grouped into three categories: *a. Speech expression*, *b. naming* and *c. comprehension*; **(C) Visuo-spatial functions**: Problems in spatial thinking may among others be manifested by *impaired construction* (e.g. inability to copy designs) and *problems of spatial orientation* (finding one's way in familiar or less familiar surroundings); **(D) Executive functions**: These are capacities involved in the planning and regulation of goal-directed behaviour. Impairment may be reflected by diverse symptoms such as apathy and loss of initiative (or conversely disinhibition and impulsivity) and perseveration (inappropriate repetition of responses). A basic aspect of executive functioning is working memory or the ability to attend to several aspects of a task at the same time, as for instance in mental arithmetic.

(2) These can only be assessed directly using tests of cognitive function.

(3) These tests need to assess various functions independently, as far as possible.

(4) The tests must yield sufficient information and be conducted with sufficient controls such that the interpretation of any identified change can be made definitively.

Neuropsychology is another branch of psychology in which a wide range of tests of cognitive function is used. The tests are generally applied to patients with cognitive deficits caused by trauma or other insults, the requirement being to identify and quantify the precise nature of the cognitive impairment. Some of the tests used in neuropsychology have been adapted for use in psychopharmacology. However there are methodological differences between the two disciplines. In fact, in neuropsychology, patients are assessed on a one-to-one basis, and often only assessed once. In psychopharmacology, volunteers and patients are trained on the tests before the start of the study and are then tested repeatedly over a study period, often in groups. Many test procedures used in neuropsychology are then not appropriate for such clinical trials, particularly when there are few parallel forms of the tests or trained specialists are required to administer them (53, 54).

Neuropyschological assessment is a powerful aid in the detection of early dementia, but has certain limitations. Firstly, the test results will be useful only if the subject is fully cooperative. Secondly, individual variation in cognitive performance is – especially in the elderly – considerable, even among people with the same demographic characteristics (age, sex, education); therefore, a wide margin of uncertainty must be allowed before impairment can be inferred. Finally, a good deal of interpretation may be required to decide what functional disturbance(s) underlie a deviant test result (54). Automated tests of cognitive function have made large advances since the introduction of these procedures to drug development programs in the early 1980s, and have shown much benefit in terms of practicality and sensitivity. Aside from not having the limitations of many traditional tests, they are also more sensitive to changes in cognitive function.

Over the last few years, an 'age of reason' has finally begun in drug development. In contrast of the policy of 'wait and see' that pervaded drug development up until the early 1990s, most developers now wish to know as soon as possible whether the compounds they are developing have desired effects and, at the same time, whether they are also free of undesired effects. E.g. desired effects of cognitive enhancers are to improve cognitive function, while for sedatives, hypnotics and anaesthetics is to impair cognitive function. By contrast, undesired effects are cognitive impairment in compounds hoped to be free of such effects, or cognitive impairment that persists longer than is desired. Cognitive-function testing is now widely incorporated into Phase I trials, even in first-administration-to-man trials. There are obvious advantages for determining the cognitive effects of a compound early in development and the practice is gradually becoming widespread (53).

The prevalence of brain disorders affecting cognition – such as stroke and dementia – increases steadily and in a linear fashion with age. However, the age effect is relatively small for cognitive tests appealing to "crystallized" abilities, i.e., skills and knowledge acquired through education and experience, such as vocabulary and common sense. In contrast, the elderly are at a considerable disadvantage on tests of "fluid" ability, which require response to novel situations. The most affected areas are episodic memory, spatial ability and executive functions. To a large extent, the changes are determined by a slowing of information processing, rather than a loss of capacity (55). The concept of "cognitive reserve" is often invoked to explain the finding that high education appears to protect against cognitive decline(56). It is debatable to what extent such reserve must be attributed to environmental (i.e., schooling) or genetic influences (aptitude). Cognitive decline is not an inevitable outcome of ageing, and may well be the result of unrecognized pathology.

Alzheimer's disease (AD) is by far the most frequent cause of dementia increasing in prevalence from less than 1% below the age of 60 to more than 40% above the age of 85. The initial phase is marked by a progressive deterioration of episodic memory. Other impairments may be entirely absent in the beginning or consist of mild disturbances of naming and executive function. When the process advances, the impairment spreads to other divisions of memory and other domains of cognition. The diagnostic criteria of AD are summarized in **Table 2.1**. Although the patients will at first be aware of their decline, insight will gradually fade and be replaced by denial and rationalization. In due time, testing becomes almost impossible, but asking few simple tasks and questions will disclose deficits in all domains of cognition (54). Biologically speaking, Alzheimer's disease (AD) may be caused by the age-dependent and progressive accumulation and deposition of Aβ-amyloid in brain – "the amyloid cascade hypothesis" (57). Aβ peptides are cleaved from the transmembrane protein amyloid precursor protein (APP) by the proteases β-secretase and γ-secretase. The secreted ectodomain of APP released by β-cleavage is APPs β. Alternatively, α-cleavage of APP within the A β sequence thereby precludes A β generation and results in secretion of APPs α. There is considerable heterogeneity of secreted A β-peptides, but A β42 is particularly amyloidogenic and fingered as an initial culprit in the pathologic cascades leading to Mild Cognitive Impairment (MCI) followed by dementia diagnosed as AD. Proponents of this hypothesis point to the fact that amyloid deposition is necessary but not sufficient for the development of AD. Other pathologies are equally important in neurodegeneration, including the formation of neurofibrillary tangles (NFT) from hyperphosphorylated tau. In fact, NFTs are one of the major pathological hallmarks of AD. They consist of paired helical filaments (PHF), deriving from abnormally hyperphosphorylated microtubule-associated protein tau.

Physiologically, tau protein is located in the neuronal axons, in the components of the cytoskeleton, and in the intracellular transport system (58). Using monoclonal antibodies, ELISA assays were developed to measure total tau protein concentration in the cerebrospinal fluid (CSF) (59). Current criteria for the diagnosis of high probability AD require both deposition of amyloid plaques and neurofibrillary tangles in brain (60).

Vascular dementia (VaD) is the second most common type of dementia, but actually consists of a heterogenous collection of clinical pictures (61). Clinical subtypes of VaD can be defined by the size of the involved vessels (large or small) or the areas of brain involved. Large-artery syndromes such as multi-infarct dementia normally present as discrete, progressive chains of events with neurologic decline occurring in steps.

In contrast, small-artery syndromes tend to develop more slowly and insidiously and include lacunar infarcts (may include apathy, slow processing, psychomotor retardation, bradykinesia, disorientation, impaired memory, inattention, and perseveration) and a condition known as Binswanger's disease which encompasses more severe symptoms than the lacunar ones (which include abulia, incontinence, and limb rigidity). The area involved on brain imaging can also predict VaD findings. Patients with lacunar infarcts tend to have progressive focal deficits and frontal-lobe symptoms. Thalamic injury promotes memory and sensorimotor deficits. Parietal strokes are associated with aphasia or visuospatial disturbance. Frontal damage is associated with behavior and executive deficits. Multiple strokes show stepwise, multiple patterns. AD and VaD symptoms may overlap, but in their pure conditions the two conditions have markedly differing presentations. In fact, executive function and focal neurologic dysfunction occur earlier in VaD. Agnosia, visuospatial problems, and short-term memory loss, occur earlier in AD (62).

In general, several systems of diagnostic criteria have been devised for VaD as an entity and these are listed in **Table 2.1**.

Table 2.1. Diagnostic criteria of AD, VaD, Mixed dementia and other dementias

Diagnosis	Criteria
Alzheimer's disease (AD) (NINCDS-ADRDA)	Development of multiple cognitive deficits, with both memory impairment and one (or more) of the following cognitive disturbances: Aphasia (language disturbance) Apraxia (learned motor skills disturbance) Agnosia (visuospatial/sensory disturbance) Executive functioning (foresight, planning, insight anticipation) Significant impairment in social or occupational functioning, representing a significant decline from a previous level of functioning Other diagnostic criteria: Hachinski Ischemic Score, ICD-10; DSM-IV; ADDTC.
Source: (63)	
Vascular Dementia (VaD) (NINDS-AIREN)	Cognitive decline from previous higher level of function in three areas of function including memory. Evidence of cerebrovascular disease by examination Evidence of cerebrovascular disease by neuroimaging Onset either abrupt or within three months of a recognized stroke.
Source: (64)	
Vascular Dementia (VaD) (Modified Hachinski Ischemia Score: ≥4)	Two-point items Abrupt onset History of stroke Focal neurologic symptoms One-point items Stepwise deterioration Somatic complaints History of hypertension Emotional incontinence Other diagnostic criteria: ICD-10; DSM-IV
Source: (65)	
Mixed Dementias (MDs)	
Hachinski Ischemic score	Score based on clinical features: ≤4=AD; ≥7=VaD; intermediate score of 5 or 6 = MD.
ICD-10	Cases that met criteria for VaD and AD
DSM-IV	Cases with criteria for primary degenerative dementia of the Alzheimer type and clinical or neuroimagery feature of VaD.
ADDTC	Presence of ischemic vascular disease and a second systemic or brain disorder.
NINDS-AIREN	Typical AD associated with clinical and radiological evidence of stroke.
Other Dementias	
Fronto-Parietal Dementia (FTD)	Behavioral or cognitive deficits manifested by either: **(1)** Early and progressive personality change, with problems in modulating behavior; inappropriate responses/activities. **(2)** Early and progressive language changes, with problems in language expression, word meaning, severe dysnomia. Deficits represent a decline from baseline and cause significant impairment in social and occupational functioning. Course characterized by gradual onset and continuing decline in function. Other causes (eg, stroke, delirium) are excluded
Source: (66)	Gradual onset and progressive cognitive decline.

(Cont'd)

Dementia with Lewy Bodies (DLB) (Consensus Guidelines for the Clinical Diagnosis for Dementia with Lewy Bodies) *Source*: (67)	Fluctuating in cognitive performance: Marked variation in cognition or function, or episodic confusion/decreased responsiveness. Visual hallucinations: Usually well formed, unprovoked, benign. Parkinsonism: Can be identical to Parkinson's Disease (PD), milder or symmetric.
PD-D (Common findings) *Source*: (62)	Bradyphrenia (slowness of thought) Executive impairment Neuropsychiatric symptoms Dysphonia

Sources: (62, 68)

The brain lesions of AD – namely, extracellular amyloid plaques and intracellular neurofibrillary tangles – and VaD – namely, cerebral infarctions, multiple lacunar infarctions, and ischemic periventricular leukoencephalopathy – often occur together (69-71). Autopsy studies indicate that in fact coexisting vascular pathology occurs in 24% to 28% of AD cases. Higher proportions (as high as 45%) were shown for community-based autopsy studies (69). As with other aspects of geriatric practice, the search for a single unifying diagnosis to explain symptoms and signs, also known as the Occam's razor rule, likely does not apply to older patients who are at risk for neurodegeneration from both AD and cerebrovascular disease. Diagnosis and treatment of co-existing AD and cerebrovascular disease is made more complicated by the absence of consensus and debate over clinical criteria and terminology. While the NINDS-AIREN use the terminology "AD with cerebrovascular disease", the other systems of classification as shown in **Table 2.1** (ICD-10; DSM-IV; Hachinski Ischemic score; and ADDTC) include a "mixed dementia" category with differing criteria. Although the term mixed dementia could include AD with other dementias (particularly PD-D and DLB), the most common use of the term is for the co-existence of AD with VaD (64, 65, 72-75).

Vascular pathology that has been associated with AD includes: Cerebral amyloid angiopathy (CAA), microvascular degeneration (tortuosity, fibrohyalinosis, liphyalinosis), disorders of the blood-brain barrier, white matter lesions and a combination of microinfarctions, lacunes, and cerebral hemorrhages (76).

Although AD, VaD and mixed dementia (MD) are thought to be the most common causes of dementia in the general population, other causes of dementia which are relatively of rare occurrence include: Frontotemporal dementia (FTD), Parkinson's disease and Parkinson Plus syndrome (PD-D and PD-Plus), Dementia with Lewy Bodies (DLB), Progressive supranuclear palsy (PSP) and Huntington's disease among others. **Table 2.1** shows diagnostic criteria of some of the other dementias for the purpose of comparison with AD and VaD diagnostic criteria.

Dementia is relatively frequent in the elderly population and was shown to affect about 6.4% of European subjects over the age of 65 years (77). A review of 50 original articles published between 1989 and 2002 using international data showed that prevalence of dementia for the very old group (85 years and over) varied between as low as 16.7% in China (78) and as high as 43% in Germany (79). This diversity was also reflected within separate age groups among the very old, ranging from 9.6% to 32% for the 85-89 age category and from 41% to 58% for the 95+ age group. In terms of incidence, the range was between 47 and 116.6 per 1000 and a separate meta-analytic study estimated the incidence in that age group to be around 104 per 1000 (80, 81). The relative prevalence of AD and VaD and the mixed version of both remains debatable. Ott and colleagues (82) estimated that for the very old, the prevalence of AD was 26.8% while that of VaD was 4.4%. However, VaD appears to be more frequent than AD in certain Japanese and Chinese populations (83).

Table 2.2 shows estimates of prevalence and incidence of AD, VaD and Mixed Dementia (MD) as well as undifferentiated diagnosis between VaD and MD using data from diverse populations.

It is worth noting that the cognitive pathology has not traditionally included impairments to *attention*. This is evident from the DSM-IV criteria for AD (74) as well as the Alzheimer's Disease Assessment Scale (ADAS-Cog) which is meant to assess efficacy of cholinergic treatment in AD. Paradoxically, it has long been known that demented patients, including those with AD, show impairments in tests of sustained and selective attention (84) as well as divided attention (85). It is also strange that a diagnostic scale such as ADAS-Cog has not incorporated attentional deficits as the cholinergic involvement in the control of human attention was first demonstrated in the late 1970s (86). Three subgroups of dementia mainly Huntington's Chorea , vascular dementia and Dementia with Lewy bodies (DLB) have been shown to have attention deficit as a hallmark symptom in their pathology. In particular, DLB is a disorder with more marked cholinergic deficits when compared to AD.

As populations age, all cognitive disorders, including dementia, become more common. However, older persons can develop demonstrable cognitive impairment, especially memory deficits, without crossing the threshold of dementia. This condition has been termed "mild cognitive impairment" (MCI) (87, 88). Hence, MCI represents a transitional state between the cognitive changes of normal aging and very early dementia and is becoming recognized as a risk factor for Alzheimer disease (AD). Although memory deficits are key in the diagnoses of both MCI and Alzheimer's disease, many researchers believe using this dimension in isolation to be too restrictive for capturing age-associated cognitive decline. The term mild cognitive impairment is reserved for patients whose impairment is objectively demonstrable but is not pronounced in more than one domain of cognition and does not seriously affect activities of daily living (89).

Several criteria have been formulated to define MCI, but the most widely used one is that for 'amnestic' MCI developed by Petersen and colleagues (88). These are aimed at the detection of isolated memory impairment that may be indicative of developing Alzheimer's disease. The advent of symptomatic treatments for Alzheimer's disease (AD) and some other types of dementia has spurred interest in the identification of the disease along with a spectrum of aging related cognitive disorders that represent prodromes of AD, particularly Mild Cognitive Impairment (MCI).

Table 2.2 Prevalence (%) and Incidence (%) of Alzheimer's Disease (AD), Vascular Dementia (VaD) and Mixed Dementia (MD) and undifferentiated VaD/MD.

Population	Source	Age	Follow-up (years)	All causes	MD	VaD	AD	VaD/MD	N
Prevalence proportion									
London, United Kingdom	(90)	≥65	n/a	n/a	0.7 (5)	0.1 (1)	3.1 (22)	n/a	705
Stockholm, Sweden	(91)	≥75	n/a	n/a	0.2 (3)	2.9 (52)	6.4 (116)	n/a	1,810
Honolulu, Hawaii	(92)	71-93*	n/a	n/a	1.4 (53)	1.8 (68)	2.1 (77)	n/a	3,734
Framingham, United States	(93)	61-93	n/a	n/a	0.2 (5)	0.4 (8)	2.3 (50)	n/a	2,180
Gothenburg, Sweden	(94)	≥85	n/a	n/a	n/a	n/a	13.0 (64)	14.0 (69)	494
New York, United States	(95)	75-89	n/a	n/a	n/a	n/a	8.4 (37)	5.7 (25)	442
Aichi Prefecture, Japan	(96)	≥65	n/a	n/a	n/a	n/a	2.4 (75)	2.8 (87)	3,106
Kanagawa Prefecture, Japan	(97)	≥65	n/a	n/a	n/a	n/a	1.2 (22)	1.6 (29)	1,800
Shanghai, China	(98)	≥65	n/a	n/a	n/a	n/a	2.6 (103)	1.1 (43)	3,888
Incidence proportion									
Gothenburg, Sweden	(94)	85-88	3	9.0	0.4	3.7	n/a	n/a	494
Stockholm, Sweden	(91)	≥75	3.5	4.3	0.2	0.7	n/a	n/a	1,810
New York, United States	(95)	75-85	8	3.4	1.0	**	n/a	n/a	442
Multiple communities, United States	(99)	≥65	5.7	14.0	n/a	n/a	n/a	6.3	3,375

Sources: (62, 68); * Men only; ** Rate not given.

However, historically speaking, MCI has been plagued by the issue of alternative conceptualization as well as multiple operationalization. In fact, in 1962, Kral defined 'Benign Senescent Forgetfulness' (BSF) as the inability to recall on certain occasions relatively unimportant parts of past experiences (100). Kral characterized BSF as an age-related problem that did not cross the boundary of disease, although it was suspected to be a transitional stage towards 'malignant' senescent forgetfulness.

Between 1968 and 1971, the word senility was rejected and replaced by dementia, to avoid the redundancy of the term with normal aging. In 1986, Crook and colleagues (101) developed specific criteria for 'Age-Associated Memory Impairment' (AAMI): age ≥ 50 with gradual onset memory complaints substantiated by relatively poor performance on neuropsychological tests (1.0 SD below mean of test value, normed on young adults). During the late 1980s and the 1990s, the AAMI criteria were criticized for the following reasons: First, they were thought to be over-inclusive; second, they were difficult to operationalize in epidemiologic studies; third, they lacked both construct and predictive validity; and finally, they did not necessarily represent decline. In 1989, Blackford and LaRue (102) altered the Crook criteria by adding the upper age limit of 79 years, requiring standardized self-report memory questionnaires, and using results on a battery of four or more tests of secondary memory to define categories of impairment based on both young adult and age-matched norms. They also required preserved general intelligence and revised the exclusion criteria. Between 1994 and 1997, Ebly and colleagues (103) and Graham et al. (104) coined the term 'Cognitive Impairment, No Dementia' which includes a classification proposed in the Canadian Study of Health and Aging (105). The CIND category was meant to encompass a variety of conditions which, while giving rise to cognitive impairment according the DSM-IIIR criteria following clinical assessment and neuropsychological testing, did not meet the criteria for dementia. Between 2000 and 2003, CIND was found to be a heterogenous group even though it was found to increase the risk of progression towards dementia. Hogan and Ebly (106) found that such progression was fastest in rate among a more narrowly defined group of CIND to whom the name MCI was given. MCI was defined as memory impairment, intellectual decline and a personality change, with no functional impairment.

The risk of progression to dementia among MCI group was found to be 55%. Fisk and colleagues (107) evaluated outcomes for a variety of definitions of MCI that either incorporated or did not include a subject memory complaint and the presence of functional impairment. Although the prevalence of MCI changed significantly with changing criteria (from 1% to 3%), rate of progression to dementia did not change appreciably per year (8-10% per year over 5 years). **Table 2.3** gives the detailed criteria used for differential diagnosis of "Mild Cognitive Impairment" along the changing descriptive terminology and classification systems.

Although many of the above terms remain in use, none have received more attention than the term MCI. Petersen and colleagues (108) was the first to give MCI a formal definition: "Having a complaint of defective memory, normal activities of daily living, normal general cognitive functioning, abnormal memory function for age, and absence of dementia." A consensus conference on MCI concluded that while MCI represents a high-risk stage for the development of AD, its heterogeneity requires sub classification: *amnestic* MCI focuses on memory loss and may progress to AD; MCI with slight impairment in multiple domains may represent normal aging or may progress to AD or vascular dementia; and MCI with impairment of a single non-memory domain may have a wide variety of outcomes. In addition to the clinical, functional and psychosocial implications, it has been noted that if patients indentified with MCI were successfully treated so as to delay progression to AD by only one year, the dollar cost savings would be quite substantial (109). Given this, increased understanding as to the clinical and neurobiological aspects of MCI, as well as the current status of potential therapeutic interventions will allow clinicians to better detect and manage this syndrome.

Prior studies indicate that the prevalence rates for MCI and other related conditions could range between 3.2% and 53.8% depending on the characteristics of the cohort as well as the screening instruments used (104, 110-112). Many potential factors for disease progression have been identified, including informant report of functional deficits of which the patient is unaware (113), EEG pattern changes (114), brain MRI imaging for volumetric measurements (115) and magnetization ratios (116), cerebral glucose metabolism (117) and cerebrospinal fluid markers (118, 119). No one factor or combination of factors has yet emerged as a clear predictor. Applying the current criteria for MCI, individuals diagnosed with MCI converted to AD at an annual rate of 12-15%, compared with normally aging individuals who convert to AD at lower rates of 1-2% per year. For longer periods of time of follow-up, MCI individuals convert to AD at a rate of 50% after 3-4 years, and a rate of 80% after 6 years (88, 109). Given the high rates of conversion between MCI and AD, some researchers contend that MCI is not nosologically separate from AD, but rather a prodrome of it (120).However, others suggest that there is a heterogeneity within MCI and that not all necessarily progress to AD when followed up for a sufficient period of time (121). However the issue of whether MCI is a prodrome or a risk factor will continue to be a source of continuous debate. Prevalence of dementia in epidemiological studies is reported at 0.3 to 1.0 per 100 people in individuals aged 60 to 64 years, and increases to a range from 42.3 to 68.3 per 100 people in individuals 95 years and older (122). Incidence rises from 0.7% per year in subjects aged 65 to 69 years to 6.6% per year in populations older than 90 years (123). Alzheimer pathology is the underlying cause of 50-70% of clinically diagnosed senile dementias (124).

Identification of subjects with MCI is gaining importance as early prevention measures and pharmacological interventions for dementia emerge. However, some disagreement remains regarding identification of MCI. The biggest practical problem has been the absence of a 'gold standard' for evaluating correctness of MCI as a dementia prodrome. Patients with MCI do not die from this mild condition, so there are no established neuropathological criteria similar to those available for AD and VaD which would provide final proof for a given clinical case of MCI (109). In characterizing MCI at a gross level, the operationalized criteria that have been proposed involve a score of 24 or more on the Mini-Mental Status Examination (MMSE) (125), a score of 0.5 on the Clinical Dementia Rating (126), and an objective memory deficit (e.g., an impaired score on paragraph recall task) (127).

There is debate of whether neuropsychological studies can actually predict dementia or identify at-risk individuals. Longitudinal investigations of elderly community-based samples indicate that preclinical manifestations of AD may appear 5-10 years prior to reaching threshold for dementia (128, 129). Longitudinal studies afford the best chance at determining which test (or tests) should be included in a battery in order to detect dysfunction as well as high risk of progression (109). For example, Ritchie and colleagues (110) used a computerized battery assessing primary and secondary memory (verbal and visuospatial memory), language skills (naming, fluency, and syntax comprehension), visuospatial performance (ideational, ideomotor, and constructional praxis, visual reasoning, form perception, and functional and semantic categorization of visual data), and focused and divided attention (in both auditory and visual modalities).

At 3 years follow-up, they found that the tests which were able to differentiate normal subjects form those with preclinical dementia two years before conversion were reaction time (on simple and dual attention task); semantic category fluency, and recall of names among others although the most predictive tests were delayed free verbal recall and delayed cued verbal recall. The finding that delayed verbal recall was the most accurate measure is consistent with results from other studies (130, 131). These results highlight the need to assess cognitive decline in these three domains and determine ways to prevent this decline. Given this fact, a number of cohort studies have been conducted using short screening tools that investigated these domains among others. A review of epidemiological studies which aimed at studying cognitive decline in the general population was conducted (132). The neuropsychological tests used in these studies and their characteristics are summarized in **Table 2.4** and examples from more recent studies and other tests were added as well.

Table 2.3 Selected diagnostic and descriptive terminology for mild cognitive impairment in older people and components of different systems of classification [2]

Acronym	Reference	Components							
		Memory Impairment	Other cognitive impairment	Cognitive decline	Subjective memory loss	Recommended cognitive tests	Associated Neurological disturbance	Associated mood disturbance	Impaired ADLs
BSF	*(100)*	*√S*	*×*	*×*	*×*	*×*	*n/s*	*n/s*	*n/s*
LCD	*(133)*	*√O*	*×*	*√S*	*√*	*×*	*n/s*	*n/s*	*×*
AAMI	*(101)*	*√O*	*×*	*√S*	*√*	*√*	*×*	*×*	*n/s*
MD	*(134)*	*√O*	*√O*	*×*	*×*	*√*	*n/s*	*n/s*	*√*
QD	*(126)*	*√O*	*√O*	*√O*	*n/s*	*×*	*n/s*	*n/s*	*√*
ARCD	*(74)*	*√S*	*n/s*	*n/s*	*n/s*	*×*	*n/s*	*n/s*	*n/s*
MND	*(74)*	*√O*	*√O*	*√O*	*×*	*×*	*√*	*n/s*	*√*
CIND	*(104)*	*√O*	*n/s*	*n/s*	*×*	*×*	*n/s*	*n/s*	*n/s*
MCI	*(88)*	*√O*	*×*	*n/s*	*×*	*×*	*×*	*×*	*×*

Source: (135)

[2] BSF: Benign Senescent Forgetfulness; LCD: Limited Cognitive Disturbance; MCD: Mild Cognitive Disorder; AAMI: Age-Associated Memory Impairment; MD: Minimal Dementia. ; QD: Questionable Dementia; ARCD: Age-Related Cognitive Decline; MND: Mild Neurocognitive Disorder; CIND: Cognitive Impairment, No Dementia; MCI: Mild Cognitive Impairment; *√O: Yes, objective; √S: Yes, subjective; √: Yes; ×: no; n/s: not specified.*

Table 2.4 Neuropsychological tests used to screen for *cognitive decline* in epidemiological studies[3]

Neuropsychological test /battery used	Key points	Studies using this battery	Study cohort name
GLOBAL & BRIEF COGNITIVE TESTS			
Mini Mental State Examination (MMSE) *Source*: (125)	• Easy to use • Tests attention, praxis, language, memory, concentration • Ceiling and floor effects • Used internationally • Reliability in assessing change over time shown for over 1 year of follow-up.	(136) (137) (138) (139) (140) (141) (142)	MoVIES Zutphen ECA LASA Chicago south-side census study Rotterdam Study Rancho Bernardo Study
Modified MMSE (MMMS or 3MS) *Source*: (143)	• Brief, general cognitive battery. • Components for orientation, concentration, language, praxis, and immediate and delayed memory. • Maximum score of 100. • More sensitive than MMSE, especially for MCI.	(144)	ABC
Telephone Interview for Cognitive Status (TICS) *Source*: (145)	• Modeled on the MMSE. • Strong linear relationship between the two tests was reported.	(146)	NHS
Wechsler Adult Intelligence Scale (WAIS and WAIS-R). *Source*: (147, 148)	• Some components shown to be highly sensitive to early change in cognition. • Used internationally since 1945. Practice effects reported. • High test-retest reliability and construct validity.	(149) (44, 150)	Dutch Longitudinal Study ARIC

[3] MoVIES: Monongahela Valley Independent Elderly Survey; ECA: Epidemiologic Catchment Area study; LASA: Longitudinal Aging Study Amsterdam; ARIC: Atherosclerosis Risk in Communities; EPESE: Established Population for Epidemiological Study; MONICA: multinational monitoring of trends and determinants in cardiovascular disease; ABC: Health, Aging and Body Composition; NHS: Nurses' Health Study; NSHD: National Survey of Health and Development; RCT: Randomized Controlled Trial; BLSA: Baltimore Longitudinal Study of Aging.

Cambridge Cognitive Examination (CAMCOG) *Source*: (134, 151)	• Designed to assess rate of decline. • Test orientation, language, memory, attention, praxis, calculation, abstract thinking and perception. • It is a self-contained part of the CAMDEX or Cambridge Examination for Mental Disorders of the Elderly • Includes National Adult Reading Test. • No ceiling effect. • Concise, but takes longer than MMSE.	(152)	Rotterdam Study
Short Portable Mental Status Questionnaire (SPMSQ) *Source*: (153)	• Test short and long-term memory, orientation, knowledge of current events and serial substraction. • Validated against clinical functional criteria.	(154)	EPESE
Geriatric Mental Schedule (GMS) *Source*: (133)	• It is a first stage cognitive screening test for global performance, like MMSE. • Usually, subjects who screen above zero on that schedule and below 26 on the MMSE are given additional domain-specific cognitive tests.	(141)	Rotterdam Study
Cognitive Function Scanner (CFS) *Source*: (155)	• Computerised Tests. • Learning, retrieval, short-term memory and retention, visuomotor and visuospatial function, concentration, visual and auditory perception, attention and vigilance.	(155)	MONICA
Blessed Dementia Scale (BDS) *Source*: (156)	• Assesses concentration by having the participant name the months of year backward. • Assesses memory by asking participants to recall a five-part name and address following a 10-minute delay. • The maximum possible score across the two items is 7.	(142)	Rancho Bernardo Study
LEARNING & MEMORY-RELATED COGNITIVE TESTS			
Audio-Verbal Learning Test (AVLT) *Source*: (157)	• Measures immediate and delayed word recall. • Uses a list of 15 words that have to be recalled immediately as well as 20 minutes later.	(139)	LASA

East Boston Memory Test (EBMT) *Source*: (158)	• Measures immediate and delayed recall of ideas. • Usually asks to recall 12 ideas in the East Boston story, immediately after it is told and after a certain period of time. • Scores ranges between 0 and 12.	(140, 159) (146)	Chicago south-side census study NHS
Buschke-Fuld Selective Reminding Test *Source*: (160)	• Assesses short and long-term storage and retrieval of spoken words. • Ten unrelated words are read to participants at a rate of one every second. • Immediately after, the participant is asked to recall the entire list. • This procedure is followed for six trials. • Measures of long and short-term memory and total recall are obtained. • Higher scores on the short-term memory test indicate poorer performance.	(142)	Rancho Bernardo Study
Story Paragraph *Source*: (161)	• Subjects are read one brief story paragraph from the Wechsler Memory Scale or an equivalent paragraph, and asked to recall as many details as possible: immediately and 40 minutes later. • Scoring of number of 'verbatim' and 'idea' units follows the Abikoff et al. method.	(162)	RCT on effect of six anti-hypertensive medications on cognitive performance.
Ryan's Verbal Paired-Associate Learning Test *Source*: (163)	• 12 unrelated word pairs (e.g. gate/native) are presented to participants (in different orders) on each of four learning trials. • Following each presentation, subjects are shown the first word of each pair as a cue and asked to name the word that was paired with it. • Delayed recall is assessed unexpectedly ~40 minutes later.	(162)	RCT on effect of six anti-hypertensive medications on cognitive performance.
Digit-Symbol Incidental Recall *Source*: (164)	• Participants are unexpectedly asked to recall the nine digit-symbol pairings from the coding scheme of the Digit-Symbol Substitution Test immediately following the test.	(162)	RCT on effect of six anti-hypertensive medications on cognitive performance.

Heaton Visual Reproduction Test *Source*: (165)	• Assesses memory for geometric forms. • Three stimuli of increasing complexity are presented one at a time, for 10 seconds each. • The participant is asked to reproduce the figures immediately to assess short-term memory; • He is also asked to reproduce them after 30 minutes of unrelated testing to assess long-term memory for geometric forms. • Afterwards, he is asked to copy the stimulus figures to assess for existing visuo-spatial impairment. • Three scores are obtained: immediate recall, delayed recall and copying.	(142)	Rancho Bernardo Study
NSHD word-learning task *Source*: (166)	• Assesses verbal memory • 15-word list is shown. Each word is shown for two seconds. • The subject is asked to write down as many words as possible. • The number of words recalled over three identical trials is summed. • Maximum score: 45	(166)	NSHD, British 1946 birth cohort
Delayed Word Recall Test *Source*: (167)	• Assesses verbal learning and recent memory. • Requires from the respondent to recall 10 common words after a 5-minute interval during which another test is administered. • The ten words used in ARIC were: chimney, salt, harp, button, meadow, train, flower, finger, rug and book. • To standardize the elaborate processing of words to be recalled, individuals are asked to compose sentences with the words that are presented. • Test scores may range between 0 and 10 words recalled and the time limit for recall is set at 60 seconds.	(44, 150)	ARIC

VERBAL FLUENCY-RELATED COGNITIVE TESTS			
Verbal Fluency Test *Source*: (168)	• Subjects are asked to name as many animals as they can in one minute.	(146)	NHS
Category Fluency test *Source*: (169)	• It is assessed by naming as many animals as possible in 1 minute. • The score is the number of animals named correctly.	(142)	Rancho Bernardo Study
Word Fluency Test *Source*: (170)	• The subject is asked to record as many words as possible using the letters F, A and S and to construct these words. • The subject is given only 60 seconds per letter. • The total score corresponds to the total number of words written in the three columns (each column corresponding to one letter). • There is no maximum score.	(44, 150)	ARIC
PERCEPTUAL SPEED-RELATED COGNITIVE TESTS			
Coding Task *Source*: (171)	• Measures information processing speed. • Three identical trials, each lasting 1 minute. • In each trial, respondent has to combine 2 characters according to a given example. • The score on each trial consists of the number of completed characters.	(139)	LASA
Symbol-Digit modalities test *Source*: (172)	• Measures perceptual speed. • Subject match as many digit-symbol pairs as possible in 90 seconds.	(140, 159)	Chicago south-side census study
Digit-span backwards test *Source*: (146)	• Subjects are asked to repeat backwards increasingly long series of digits. • Total of 12 series. • Scores can range between 0 and 12.	(146)	NHS

Digit Span test *Source*: (148)	• Seven pairs of random number sequences of increasing length are presented orally to subjects. • Subjects are asked to simply repeat each number sequence (Digits forward) or repeat the sequences in reverse order (Digits backward)	(162)	RCT on effect of six anti-hypertensive medications on cognitive performance.
NSHD visual search task *Source*: (166)	• Each subject is required to cross out the letters P and W, randomly embedded within a page of other letters, as quickly as possible within one minute. • Scores are computed as total number of letters searched (maximum is 600) minus the number of targets missed.	(166)	NSHD, British 1946 birth cohort
Trail Making test, part A *Source*: (173)	• Subjects are asked to draw a line connecting a series of consecutive numbers as fast as possible. • The time of the completion of the task is recorded.	(162) (174)	RCT on effect of six anti-hypertensive medications on cognitive performance. BLSA
Trail-Making test, part B *Source*: (173)	• Tests visuo-motor tracking and attention. • The participant scans a page continuously to identify numbers and letters in a specified sequence while shifting from number to letter sets. • A maximum of 300 seconds is given. • Scoring is the time taken to finish the test. • A higher score indicates a poorer test performance	(142) (162)	Rancho Bernardo Study RCT on effect of six anti-hypertensive medications on cognitive performance.
Digit-Symbol Substitution Test *Source*: (148)	• Subjects are presented a coding scheme in which single-digit numbers (1-9) were each paired with a unique symbol. • On a grid of random number, participants use the answer grid to write the correct symbol below each number as quickly as possible. • The response measure is the number of symbols completed correctly in 90 seconds.	(162) (44, 150)	RCT on effect of six anti-hypertensive medications on cognitive performance. ARIC

Two screening instruments that have been widely used for the purpose of global cognitive impairment screening are MMSE and Cognitive Capacity Screening Examination (CCSE) (125, 175). It was noticed that MMSE was too biased on verbal items as compared to CCSE and that CCSE was a more sensitive screening tool especially in detecting right hemisphere related dysfunction (176). However, CCSE was heavier on abstract test items and hence was highly influenced by educational attainment (177). Three testing components, namely orientation, word recall, and serial sevens, overlap between CCSE and MMSE that involve 13 questions (13 points). For other domains, different test items are used, with MMSE putting emphasis on language items (8 points) and CCSE emphasizing digit items (15 points). **Table 2.5** summarizes the items found in each of these cognitive screening tests along with their scoring system.

Other cognitive scales that are commonly used to assess global cognitive impairment or decline through a relatively short interview include ACL -- Allen Cognitive Levels, GDS -- Global Deterioration Scale, ADAS -- Alzheimer's Disease Assessment Scale, BCRS -- Brief Cognitive Rating Scale, NCSE -- Neurobehavioral Cognitive Status, and CDR – Clinical Dementia Rating. Each one has its own scoring system as well as diagnostic criteria for cognitive impairment severity and type of dementia, if applicable.

A consensus conference suggested the following criteria to be used in order to judge the quality of an AD biomarker: (1) It should be able to detect a fundamental feature of AD neuropathology, validated in neuropathologically confirmed AD cases; (2) It should be precise (able to detect AD early and distinguish it from other dementias); (3) It should be reliable, noninvasive, simple to perform, and inexpensive; (4) Ideally, it should have a clear separation between the normal and abnormal range of values.

Since the cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the central nervous system, biochemical changes in the brain could potentially be reflected in CSF. Therefore, CSF constitutes a potential source for clinically useful biomarker of AD. By the criteria cited above, the combination of increased [tau] and decreased [A β_{42}] in CSF is a leading candidate as a biomarker of AD. Both of these protein alterations were consistently observed among AD patients. However, lumbar puncture which is a pre-requisite to obtain CSF is a highly invasive procedure and unlikely to be used as a standard practice (58).

Table 2.5 Scoring systems of MMSE and CCSE cognitive screening tools

MMSE		CCSE	
Domain	Points	Domain	Points
Visuo-spatial & Executive domains		*Visuo-spatial & Executive domains*	
Orientation to time	5	Orientation to time	4
Orientation to place	5	Orientation to place	1
Subtracting serial sevens from 100 or backward spelling (*highest score of the two*)	5	Digit span	2
		Reversing of digit span	1
		Reversing the sequence of days of week	1
		Calculation	3
		Subtracting serial sevens from 100	7
Memory domains		*Memory domains (highest score of the two)*	
Registration of three words	3	Recalling digits	2
Delayed verbal recall	3	Word Recall	4
Language domains		*Language domains*	
Naming objects	3	Word Antonym	3
Reading a sentence	1	Item classification	3
Writing a sentence	1		
Compliance with three-step command*	3		
Copying a spatially arranged design of figures*	1		
Highest possible score	30		30
Diagnostic Criteria for both tests: **24 - 30** normal, depending on age, education, complaints **20 - 23** mild **10 - 19** moderate **1 - 9** severe **0** profound			

Sources: (125, 175); *Comprehension sub-domains of language

A study by Olsson and colleagues (178), showed that A β_{42} in CSF may particularly be able to differentiate between AD and Mild Cognitive Impairment given the dose-response relationship that was observed. Moreover, in patients suffering from MCI who converted to AD during follow-up, elevated t-tau protein levels at baseline were found in relatively small samples. Memory-impaired subjects who later progressed into manifest AD could be discriminated by high CSF t-tau protein from those who did not progress with 90% sensitivity and 100% specificity (118, 179). Extracellular SP consistent of Aβ is a histopathological hallmark for the diagnosis of AD (180). It is the source of a pathogenic protein with 42 amino acids ($A\beta_{1-42}$) (181). Unlike t-tau protein, beta-amyloid protein did not show enough evidence of being a highly sensitive and specific biomarker for progression from MCI to AD (182). Since diagnostic accuracy of CSF t-tau protein and $A\beta_{1-42}$ protein alone is not sufficient in the differentiation of AD from other clinically relevant dementias, a combination of both markers has been suggested to enhance the diagnostic accuracy of AD. However, studies did not confirm this hypothesis.

Table 2.6 Putative biomarkers of Alzheimer's disease

Neuropsychometrics
Sentence complexity
Structural neuroimaging
Medial temporal lobe atrophy or a change over time (MRI)
Amyloid ligand imaging (PET)
Functional neuroimaging
Single photon emission computed tomography (SPECT)
[^{18}F]-fluoro-2-deoxyglucose positron emission tomography (FDG-PET)
Functional MRI (fMRI)
Magnetic resonance spectroscopy (MRS)
Genomics
Presenilin-1*, presenilin-2, or APP mutations (in familial AD only)
Apolipoprotein E (ApoE) genotyping*
Proteomics
A β42*
Tau* or phspho-Tau (phosphothreonine-181 Tau*)
A β42 and Tau*
Neuronal thread protein (NTP)*
APPs α, APPs β (in this volume)
Soluble form of iron-binding protein (p97)
Glial fibrillary acidic protein (GFA)
α_1 – Antichymotrypsin

(Cont'd)

Metabolomics
Isoprostanes
24S-hydroxy-cholesterol
Nitrotyrosine
8-hydroxy-2-deoxyguanosine

Source: (58); * Commercially available biomarkers

Levels of cognitive impairment commonly found in community studies give rise to an increased risk of mortality, and this appears to be true even for quite mild levels of impairment. A systematic review of the literature on dementia, cognitive impairment and mortality in persons aged 65 and over and living in the community confirmed that there is a positive association between impairment and mortality. Out of the 68 studies that were reviewed between 1966 and 1999, 12 were carried out in the United States, and the lag period between assessment of impairment and mortality assessment ranged between 1 and 10 years for most of the studies while in some a follow-up period of 20 and 25 years was chosen. In eight studies of cognitive impairment, MMSE was used as the main screening tool and three of those used the 24 points cutoff point for mild cognitive impairment (183). The risk ratio of having a score less than 24 points as compared to having 24+ and mortality was shown to be equal to 2.0 with a 95% CI: 1.33-3.00 in one study (122), while in another study having a score lower than 24 had a weaker association with mortality: RR=1.43 (95% CI: 0.78,2.65) (184). In two studies using the Wechsler Adult Intelligence Scale (WAIS) a strong association was found for the overall score, although a weaker but significant association was also found for the digit symbol subscale of WAIS (185, 186). This weaker association in the second study may be due to adjustment for confounding with age and education. In general, the studies of cognitive impairment showed a significant association with mortality risk even after control for age, education, marital status, and various health and psychosocial variables.

Studies of dementia using DSMIII, DSMIII-R, CERAD, AGECAT, CAMDEX, and NINCDS-ADRDA criteria to detect Alzheimer's disease, all types of dementia and vascular dementia showed a positive association with mortality after control was done on age and sex. It is worth noting however that additional control for education, marital status, ADL limitation, vision and hearing disabilities lead to non-significant association between dementia and mortality risk (187). In studies which controlled only for age, sex and education, the association was still significant. Therefore, one can infer that the largest amount of confounding in the association between dementia and mortality risk has to do with functional health and other health-related variables. In a sub-set of the studies on dementia and mortality, a meta-analysis was possible and age-adjusted odds ratios were pooled to obtain an overall value of 2.63 (95% CI: 2.17-3.21) (183). The detailed results are shown in **Table 2.7**.

Table 2.7 Meta-analysis of dementia as a mortality risk factor

Study	Cohort size	Age group	Follow-up time (years)	Diagnostic criteria used	Effect size		Confounders
					OR	95% CI	
(188)	778	60+	7	DSMIII	7.88	3.71-16.75	Age & sex
(189)	2550, 1013	60+	25, 15	AGECAT	4.39	2.29-8.05	Age & sex
(190)	1080	65+	4.5	AGECAT	3.7	2.0-6.7	Age, sex & education
(191)	656	75+	1	DSMIII-R	8.45	1.78-6.72	Age
(192)	358	85+	4.7	Clinical	2.08	1.56-2.77	Age
(193)	3623	65+	4.9	NINCDS-ADRDA	1.80	1.13-2.85	Age
Pooled					2.63	2.17-3.21	

Source: (183)

Hence, an independent, inverse association between cognitive function and all-cause mortality may exist in elderly cohorts. A recent study using the Atherosclerosis Risk in Communities (ARIC) cohort data was able to prove that this effect on mortality can also be determined by cognitive functioning among middle-aged adults.

The detailed methodology used in the ARIC study will be discussed in a later section. However, it can be summarized here as follows: It is a cohort study initiated in 1987 to investigate the development of atherosclerosis in middle-aged persons. Three cognitive function measures were included in the second cohort examination conducted from 1990 to 1992 when the participants were aged 48–67 years: the Delayed Word Recall Test (DWRT), the Digit Symbol Substitution Test (DSST) (a subtest from the Wechsler Adult Intelligence Scale-Revised), and the Word Fluency Test from the Multilingual Aphasia Examination. In that study, Cox proportional hazards modeling was used to determine whether all-cause mortality ascertained through 1997 was associated with each measure after adjustment for sociodemographic, biologic, psychologic, and behavioral risk factors. Without adjustment, there was a significantly lower mortality hazard associated with higher scores on all three measures. After covariate adjustment, the hazard ratios for the DWRT and the DSST remained significant (hazard $\text{ratio}_{\text{1-point DWRT score increment}} = 0.90$, 95% confidence interval: 0.84, 0.97; hazard $\text{ratio}_{\text{7-point DSST score increment}} = 0.86$, 95% confidence interval: 0.80, 0.93). Hence, cognitive function measured in middle age appears to have prognostic importance for life expectancy similar to that reported in elderly adults (194). Based on the previous discussion a time line for cognitive events can be drawn as shown in **Figure 2.1**.

In summary, cognitive function which refers to mental processes that are crucial for the conduct of activities of daily living has been measured both globally and in terms of separate domains. These domains have been grouped under memory, language, visuo-spatial functions and executive functions. Neuropsychological assessment is a powerful aid in the detection of early dementia and can be used to screen for mild cognitive impairment (MCI) a now well-proven prodrome for Alzheimer's Disease (AD). However, new studies prove that AD and other types of dementia are often co-morbid which generated the concept of mixed dementia.

In addition, MCI has been historically plagued by the issue of alternative conceptualization as well as multiple operationalization. Nevertheless, identification of subjects with MCI is gaining importance as early prevention and pharmacological interventions for dementia emerge. A number of biomarkers are being developed which can aid in accurate prediction of potential conversion from MCI to AD. Finally, cognitive impairment and dementia have been shown to be independent risk factors for all-cause mortality.

Cognitive development, low prevalence of decline	Stagnant cognitive development, low prevalence of decline *Risk factors* of cognitive decline are particularly modifiable during that period.	Increased prevalence of cognitive decline. Degree of decline should be age-group standardized. Prevalence of *dementias*: <1% for <60 years 0.3-1%: 60-64 years 42.3-68.3%: 95+ 55-70% of the dementias are AD.	Verbal fluency, Digit symbol substitution and *Delayed word recall* are most affected. Their decline is highly predictive of MCI as diagnosed using Petersen's (1999) criteria. MCI prevalence: 3.2% AACD prevalence: 19.3%	Prevalence of AD increases to 10% (65+). Incidence of AD increases from 0.7% (64-69years) to 6.6% per year for 90+ age group	Prevalence of AD dementia increases to 40% .
0-39 years	**40-54 years**	**55+ years**	**3 years+ follow-up baseline age 55+**	**65+**	**85+**

Age (years)

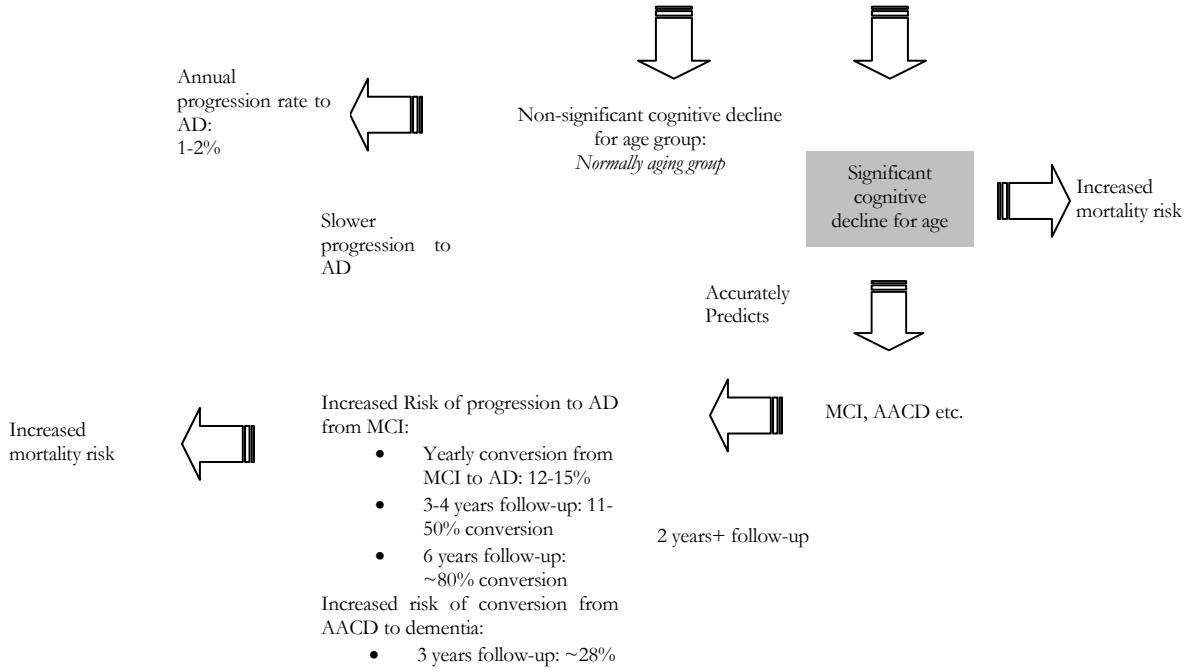


Figure 2.1 Diagram depicting timeline of cognitive events

Sources: (88, 91, 107, 109, 110, 120, 121, 123, 124, 128-131, 183, 194)

B.2. Epidemiologic studies of risk factors for cognitive impairment

The following is an account of epidemiologic studies conducted on risk factors of cognitive impairment in general, and cognitive decline in particular. Most of these studies were prospective cohort or cross-sectional, with a few exceptions where a case-control design was used. While the outcome could be impairment at a single point in time, decline over time, dementia of all types or its sub-types (AD, VaD, Mixed dementia or other dementias), risk factors were grouped under socio-economic, genetic and hormonal, behavioral and nutritional, and co-morbid conditions and medications.

B.2.1. Socio-economic factors

Early life conditions are related to cognitive development and abilities in childhood and cognitive function in adulthood. The association between early life conditions and cognitive change in old age is virtually unknown. Low educational attainment and other markers of low socio-economic position (SEP) in adulthood were associated with poorer cognitive function in adulthood and age-related cognitive decline and impairment (195-197), greater risk or prevalence of dementia and Alzheimer's disease in the elderly (198-200) and overall worse health (201). These findings support the cerebral reserve hypothesis, which is a heuristic concept used to explain apparent protection from onset of cerebral disease and/or cognitive decline in old age. A study by Staff and colleagues (202) tested three hypothesized proxies of reserve (education, head size and occupational attainment) in 92 volunteers born in 1921 whose cognitive function was measured at two points in time, namely age 11 and 79 years, and who also underwent brain MRI. Adjusting for childhood cognitive function as well as MRI age-related pathological changes, the effect of the brain reserve proxies on old age cognitive function was assessed.

Results indicated that education and occupational attainment both act to preserve cognitive function in old age, unlike total intracranial volume and each explain 5-8% of the variance in old-age functioning. A recent cohort study, however, conducted by Everson-Rose and colleagues (140) among 4,398 community-dwelling adults (62.1% female; 61.7% Black) aged 65 years or more from Chicago, Illinois suggested that socioeconomic and cognitive conditions in early life contribute to absolute levels of cognitive function but do not protect against cognitive decline in later life. Hence, the findings do not support the cerebral reserve hypothesis which states that early life conditions affect the pace of cognitive decline in later life. A cross-sectional study by Kaplan and colleagues (203) indicated that higher socio-economic position during childhood and greater education attainment are both associated with cognitive functioning in adulthood, with mothers and fathers each contributing to their offspring's formative cognitive development and later life cognitive ability (albeit in different ways). Improvements in both parental socioeconomic circumstances and the educational attainment of their offspring could possibly enhance cognitive function measured by a battery of cognitive tests (Trail making test, selective reminding test, verbal fluency test, Russel's adaptation of the Visual Reproduction test, and the Mini-Mental State Examination) and decrease risk of dementia later in life. This study agrees with the findings from Everson-Rose and colleagues (140) in that socio-economic position affects current cognitive functioning rather than decline. In contrast, Lee and colleagues (146) found otherwise. They investigated the relationship of educational attainment, husband's education, household income, and childhood socioeconomic status to cognitive function and decline among community-dwelling women aged 70-79 years. Between 1995 and 2000, six cognitive tests were administered to 19,319 Nurses' Health Study participants. Second assessments -- as of April 2002 -- were completed for 15,594 women.

On a global score combining the results of all tests, women with a graduate degree had significantly decreased odds of a low baseline score or worst 10% of the distribution (odds ratio =0.49, 95% CI: 0.36-0.66) and decline or worst 10% of decline distribution (odds ratio=0.65, 95% CI: 0.50-0.86) in comparison with women with a Registered Nurse diploma. However, much weaker associations were evident for other socioeconomic variables.

There are several possible mechanisms supporting the findings that less education is related to cognitive decline. First, education may exert direct effects on brain structure early in life by increasing synapse number or vascularization and creating cognitive reserve. This has been called the "reserve capacity" hypothesis. Second, education in early life may have effects in later life if persons with more education continue searching for mental stimulation ("the use it or lose it" hypothesis), which may lead to beneficial neurochemical or structural alterations in the brain (204). Indeed, recent mental stimulation has been associated with improved cognitive functioning in one study (205). Alternatively, education may act through several behavioral mediators to improve health in general, and cognitive functioning in particular (204). This hypothesis was confirmed by a study using the Framingham cohort which suggested that education was uniquely protective against vascular dementia and was not associated with Alzheimer's disease (206). This finding was explained by the mediating effects of other risk factors of cognitive decline, which include smoking and hypertension, which in turn can initiate cerebrovascular damage.

However, Lee and colleagues (146) disproved this hypothesis by showing a sustained strong association between education and cognitive functioning even after adjustment for behavioral and health-related factors. Aside from education, employment and type of occupation were shown to act as independent risk factors for cognitive functioning and/or decline, although studies have shown inconsistent results.

The effect of lifetime employment on risk of Alzheimer's disease, vascular dementia and dementia with Parkinsonism was investigated by Helmer and colleagues (207) among a cohort of 2,950 non-demented elderly persons (aged 65+) living at home and selected from the South West area of France. Repeated examinations after 1,3,5,8 and 10 years of follow-up with identical standardized neurological and neuropsychological measures yielded a total of 251 subjects with Alzheimer's disease, 112 with vascular dementia and 27 with dementia accompanied by Parkinsonism. Using Cox proportional hazards models, and controlling for sex, education, tobacco, and wine consumption, findings indicated no effect of occupation on the incidence of Alzheimer's disease but rather an increased risk of dementia with Parkinsonism among farmers (as compared to professionals and managerial group), particularly among women (RR: 7.47; 95% CI, 1.80-31.07). It was concluded that the effect of occupation on cognitive function may be mediated by variations in health behaviors or environmental exposures.

Finally, social engagement, defined as the maintenance of many social connections and a high level of participation in social activities, has been thought to prevent cognitive decline in later life. A recent cohort study by Bassuk and colleagues (208) was conducted in New Haven, Connecticut, among 2,812 non-institutionalized elderly persons (65+ years) and interviews completed at four points in time (namely, 1982, 1985, 1988 and 1994). A global social disengagement scale was constructed from the following indicators: presence of a spouse, monthly visual contact with three or more relatives or friends, attendance at religious services, group membership, and regular social activities. Cognitive function was assessed with the SPMSQ with categorization of scores into high, medium or low and cognitive decline defined as a transition to a lower category.

Results indicated that compared to individuals having 5-6 social ties, those having no social ties were at increased risk of cognitive decline after adjustment for age, sex, ethnicity, education, income, housing type, physical disability, cardiovascular profile, and sensory impairment, symptoms of depression, smoking, alcohol use and level of physical activity. The 3-year odds ratio was 1.91 (95% CI: 1.14-3.18); and the 12-year odds ratio was 2.37 (95% CI: 1.07-4.88). It was concluded that social disengagement is a risk factor for cognitive impairment among elderly persons.

B.2.2. Genetic & hormonal factors

Apolipoprotein E (ApoE) ε4 genotype was shown to be a risk factor for Alzheimer's disease (AD). In fact, according to a cohort study by Wilson and colleagues (209) the relative risk of developing AD by presence of an ε4 allele was 1.92 (95% CI: 1.27-2.89) and a relatively selective effect was noted for episodic memory change. Similar findings were reported by other studies (210, 211). However, its effect on predicting conversion from normal to "cognitive impairment, no dementia" (CIND) and from CIND to AD is less clear. A recent study conducted by Hsiung and colleagues (212) using data fro the Canadian Study of Health and Aging suggested that possession of the ε4 allele of the ApoE gene increased the risk of conversion from CIND to AD, even though its predictive value was not of clinical significance. However, it can be made use of in research in order to enrich samples with high-risk of conversion to AD individuals. Dik and colleagues (139) conducted a prospective cohort study on 1,224 subjects aged 62 to 85 years over a period of 3 years and assessed cognitive decline in relation to stroke and Apolipoprotein E ε4. Using several measures of cognitive performance, he concluded that stroke and Apo E ε4 may impair cognition through distinct nonsynergistic mechanisms. The slowing of information speed for ApoE ε4 was more evident than impairment in memory.

An earlier smaller size study by Kalmijn and colleagues (213) suggests that there is a synergistic effect between Apolipoprotein E ε4 and stroke in increasing the risk of cognitive decline among community-dwelling elderly men. This genotype (Apo Eε4) was not found to be a risk factor for cognitive decline (measured using the CERAD neuropsychological battery) in normal CVD-free elderly individuals according to Kim and colleagues (214) and there was no interaction with age or gender. Recently, Yip and colleagues (215) analyzed the Medical Research Council Cognitive Function and Ageing Study data and found that risk of dementia among elderly men and women possessing two ε4 alleles as opposed to those possessing none was increased considerably (OR: 3.8, 95%CI: 1.0-14.0), although this association was highly specific to AD diagnosis and was not statistically significant for other dementias. Using ARIC data, Blair and colleagues (216) found that cognitive decline was mostly seen among Caucasian men and women aged at baseline between 47-68y with at least one APOE ε4 allele and they concluded that the effect of this genotype is seen among middle-aged adults well in advance of overt dementia.

Decreased estrogen levels have been hypothesized to be associated with increased risk of dementia. A relationship between estrogen levels and dementia has been shown to be biologically plausible according to a number of studies (217-220). In fact, estrogen was shown to improve synapse formation on dendritic spines in the hippocampus of oophorectomized rats. It is also thought to improve cerebral flow and glucose metabolism and may act as an antioxidant. An alternative mechanism of action on dementia by estrogen level would be through the reduction of cardiovascular disease risk. A recent study by Geerlings and colleagues (221) aimed at determining whether a longer reproductive period, as an indicator of longer exposure to endogenous estrogens, is associated with lower risk of dementia and Alzheimer's disease (AD) in 3,601 older women (aged 55 years or more) who have natural menopause and no dementia at baseline.

After a median follow-up time of 6.3 years, 199 women developed dementia and 159 developed AD. The authors found no significant association between length of reproductive period and incidence of AD or dementia. However, among those carrying the Apo Eε4 allele, exposure to endogenous estrogen surprisingly seemed to increase the risk of AD and dementia. Although earlier research had found a protective effect of exogenous estrogen on cognitive functioning among older adults, these studies have been criticized because of methodological issues (222-224). A recent meta-analysis of twelve observational dementia studies suggested that Hormone Replacement Therapy (HRT) was associated with a decreased risk of dementia (summary odds ratio: 0.66; 95% CI 0.53-0.82). However, most of the studies included in that meta-analysis suffered from important methodological limitations (225). In fact, more up-to-date results from a large clinical trial (Women's Health Initiative Memory study) proved that in fact, exogenous estrogen has deleterious effects on cognitive functioning unlike what was shown in earlier observational studies (226).

B.2.3. Behavioral & Nutritional factors

Several behavioral risk factors have been studied in relation to cognitive performance among older adults, including smoking, alcohol drinking, caffeine consumption and physical activity. Population-based evidence of an effect of smoking on cognitive function has been inconclusive, with most longitudinal studies reporting weak or null associations (227-231). However, a number of other studies have found a positive association between smoking and risk of dementia and AD (232, 233) as well as cognitive impairment (234). A recent study by Richards and colleagues pointed to the difficulty of finding an association between smoking and cognitive impairment given the differential high mortality of smokers especially among the elderly population(166). This study used the British 1946 birth cohort.

After controlling for a range of socioeconomic and health status indicators (both physical and mental), the study found that smokers who survive into later life may be at risk of clinically significant cognitive decline. However, these effects were largely accounted for by heavy smokers i.e. those who smoked 20 cigarettes per day or more. Earlier research conducted by Kalmijn and colleagues on middle aged adults suggested that current smoking and number of pack-years of smoking were related to reduced performance on tests of psychomotor speed and cognitive flexibility assessed approximately five years later (235). This cohort study (Rotterdam study) also looked at alcohol use and as was shown in at least one other cross-sectional study (236), past alcohol consumption's effect on speed and flexibility appeared to be slightly U-shaped, with the best performance observed among those who drank 1-4 glasses of alcohol per day, although this association was stronger among women than among men. Surprisingly, a number of prior cross-sectional, cohort and twin studies had found an inverse association between alcohol consumption and cognitive impairment (237-239). Others, however, found no clear association between alcohol consumption and cognitive impairment (240). Caffeine is known to be the most widely used psychoactive drug worldwide and its main source is coffee particularly in Western diets. Acting as a stimulant of the central nervous system, it causes heightened alertness and arousal (241, 242). Previous literature yielded inconsistent findings as to the effects of caffeine consumption on cognitive processes. In fact, in some studies, caffeine was shown to improve perceptual speed and vigilance (243, 244), while in others more complex functions such as memory were shown to be impacted as well (244, 245). Caffeine is one type of compounds known as methylxanthines whose effects are mainly to block adenosine receptors in the brain, resulting in cholinergic stimulation. It has been hypothesized that such stimulation would lead to improved memory (246).

A recent cross-sectional study conducted by Johnson-Kozlow and colleagues (142) on 638 men and 890 women aged 50 years or older looked for an association between lifetime coffee consumption as well as current consumption of caffeinated and decaffeinated coffee and performance on several cognitive tests. The results were suggestive of a protective effect of lifetime and current consumption of caffeine on the cognitive functioning of women aged 80 years or more, although this effect did not reach statistical significance. Physical activity has well-known benefits for preventing a number of chronic disorders, including coronary heart disease, stroke, diabetes mellitus and osteoporosis. However, its impact on cognitive functioning has not been studied extensively. Two case-control studies examining the role of physical activity on the risk of Alzheimer's disease suggested a protective effect (78, 247). In a few prospective studies, discordant results were reported (248, 249). A recent cohort study by Laurin and colleagues (250) conducted on 4,615 men and women aged 65 years or more (the 1991-92 Canadian Study of Health and Aging) who were cognitively normal at baseline and had complete follow-up data after five years found that high levels of physical activity were associated with reduced risk of cognitive impairment (OR: 0.59; 95% CI: 0.41-0.83), Alzheimer's disease (OR: 0.50; 95% CI: 0.28-0.90) and dementia of any type (OR: 0.63; 95% CI: 0.40-0.98) even after adjusting for age, sex and education. These findings suggested that physical activity could represent an important and potent protective factor for cognitive decline and dementia in elderly persons. Similar findings were obtained by other recent prospective studies (251-253). Several mechanisms of action may underlie the potentially protective effects of physical activity on cognitive function, including sustained cerebral blood flow (254), improved aerobic capacity and cerebral nutrient supply (255, 256) and more recently growth factors, specifically brain-derived neurotropic factor, which is a molecule that increases neuronal survival, enhances learning, and protects against cognitive decline (257, 258).

Nutritional factors that were studied in relation to cognitive performance included body mass index and weight change as well as antioxidant nutrients and other potentially beneficial micronutrients. Over the years, there has been accumulated evidence that baseline weight (expressed in terms of body mass index) and weight change are both associated with cognitive performance or decline, based on three cohort studies (259-261), one intervention trial (262) and one case-control study (263). However, it is still unclear in which direction this association prevails and whether dietary restriction and weight loss can play an important role in the prevention of Alzheimer's disease. A recent prospective cohort study by Brubacher and colleagues (264) conducted on 531 healthy subjects (84% of whom were men) looked at the effect of annual change in BMI over a period of 10 years on cognitive performance at the end of the follow-up period. Using the CERAD-NAB battery to assess cognition, the authors concluded that change in body mass index (in its absolute value) was inversely related to cognitive performance, after controlling for socio-economic, demographic and genetic factors. They concluded that this association may be either a direct consequence of cognitive impairment or an early symptom of neurodegenerative disease. Several findings suggest that oxidative stress may play an important role in the pathogenesis of Alzheimer's disease. First, the brains of AD patients have lesions that are associated with exposure to free radicals. Moreover, oxidative stress among these patients is also marked by an increased level of antioxidants in the brain which act as free radical scavengers. Finally, in vitro studies suggest that exogenous antioxidants may reduce the toxicity of β-amyloids in the brains of AD patients (265-267). Based on these findings, it may be hypothesized that dietary antioxidants may help reduce the risk of AD. Epidemiologic studies have been conducted to examine the longitudinal relationship between *supplemental antioxidants* and risk of Alzheimer's disease and other dementias.

These studies found conflicting results: While vitamin C supplement use was related to lower risk of Alzheimer's disease in one cohort study (268), combined supplementation of vitamin E and vitamin C was associated with reduced prevalence and incidence of AD in another (269), whereas there was only borderline evidence of benefit from combined use of vitamin C and vitamin E supplements according to an independent cohort study (270). Several prospective cohort studies were carried out on the effect of *dietary antioxidants* on the risk of dementia. One study found a reduced risk of dementia associated with increased intake of flavonoids (271) while another found that high dietary intake of vitamins C and E may reduce the risk of Alzheimer's disease (272). The latter study found that relationship to be most pronounced among smokers. Morris and colleagues (273) found that dietary intake of vitamin E, but not other antioxidants, was associated with a reduced risk of incident AD, although this association was restricted to individuals without the APOE ε4 genotype. A recent study, however, suggested that neither dietary nor supplemental antioxidants were able to reduce the risk of Alzheimer's disease (274). Similarly, Laurin and colleagues (275) found no association between midlife dietary intake of vitamins E and C and incidence of dementia. Irrespective of the source of antioxidants, plasma concentration may be a good biomarker for oxidative stress status. A nested case-control study carried out recently by Helmer and colleagues (276) using the PAQUID cohort suggested that subjects in the lower tertile of plasma vitamin E concentrations were at higher risk of developing dementia in subsequent years than subjects in the upper tertile (OR: 3.12, P=0.033).

Aside from antioxidants, other micronutrients have been linked to cognitive functioning including the B-vitamins and folate. An elevated level of plasma concentration of the sulfur amino acid homocysteine (hyperhomocysteinemia) is now recognized as an independent risk factor for cardiovascular, peripheral vascular, and cerebrovascular disease (277).

Accordingly, a potential influence of hyperhomocysteinemia on cognitive functioning among older adults has been postulated and several studies were able to associate high levels of homocysteine with increased risk of Alzheimer's disease and dementia (278-281). A recent study by Miller and colleagues (282) showed that such an association was modest and that reducing the risk of cognitive decline through increased intake of B-vitamins which lower homocysteine levels in plasma can be limited. Moreover, results from the Rotterdam Scan Study of 472 subjects, based on MMSE scores, suggested that a drop of the score by 1 point/year or more was predicted by an increased plasma levels of homocysteine (upper tertile) with a risk ratio of 1.4. However, this association did not reach significance in the Rotterdam cohort study of cognitive decline (283, 284). Although low folate intake has been mostly implicated in the etiology of depression, depression itself has been linked to cerebrovascular disease and hence intake of folate may help reduce the risk for cognitive decline by reducing the damaging effects of depression on cerebrovascular health (285). In a cross-sectional study of middle-aged elderly men, higher plasma concentration of vitamin B_{12} ($P=0.04$) and folate ($P=0.03$), and lower plasma concentration of homocysteine ($P<0.01$) were found to be associated with better spatial copying skills, whereas performance on two tests of memory (backward digit span and incidental recall) was positively associated with plasma level of vitamin B_6 (286).

B.2.4. Co-morbid conditions & Medications

Vascular disease and its risk factors are receiving increasing attention as potentially modifiable causes of cognitive decline and dementia in older adults. Stroke, cardiovascular disease, peripheral vascular disease, hypertension and diabetes have each been associated with cognitive deficits or dementia, or both, in various elderly populations in several cohort (287), cross-sectional (288-290) and case-control studies (291). Few prospective studies have assessed diabetes mellitus as a risk factor for incident Alzheimer disease (AD) and decline in cognitive function.

A recent study by Arvanitakis and colleagues (292) examined 824 older Catholic nuns, priests and brothers annually for up to 9 years to assess their cognitive function. Diabetes was present in 15.4% of the sample and during the mean of 5.5 years of follow-up, 151 subjects developed AD. After adjusting for age, sex and educational level, the hazard ratio for diabetes and AD was 1.65 with a 95% CI 1.10-2.47. In addition, diabetes increased the risk of cognitive decline in perceptual speed by 44% (P=0.02) but not in other cognitive systems. Similar findings were replicated in a recent study by Yaffe and colleagues (293) who found that risk of developing cognitive impairment -- based on five standardized tests -- among women with Impaired Fasting Glucose (IFG) or diabetes was increased by almost twofold (age and treatment-adjusted OR = 1.64; 95% CI 1.03 to 2.61 for IFG; OR = 1.79; 95% CI 1.14 to 2.81 for diabetics). Using the EVA longitudinal study, another group of investigators compared the performance of 55 type 2 diabetes participants to the performance of 103 participants with impaired fasting glucose and 768 aged-matched controls (mean age=65 years) on several neuropsychological tests, including MMSE, measures of verbal and visual memory, facial recognition, executive function, motor function, arithmetic ability, processing speed, and logical reasoning. Although the authors controlled for age, gender, education, depression, hypertension and body mass index, they reported that diabetic participants had significantly higher levels of triglycerides and lower levels of HDL and total cholesterol as compared to controls. They concluded that the odds ratio of belonging to the worst 15% of the distribution of the whole sample was greater than 2 for measures of verbal memory, facial recognition, processing speed, and motor function when controlling for age, gender, education, and systolic blood pressure (294). Finally, Hassing and colleagues (295) in their longitudinal study of 258 elderly subjects found a significant interaction between hypertension and diabetes and the presence of both diseases tended to produce pronounced MMSE cognitive decline.

The relation between plasma lipid levels and the incidence or prevalence of Alzheimer's disease (AD) and vascular dementia (VaD) remains unclear. Cholesterol alters the degradation of the amyloid precursor protein, which plays a major role in the pathogenesis of AD (296). Moreover, cerebrovascular disease which is associated with dyslipidemia, may be related to the risk of AD (297). Previous literature showed that reduced HDL-C (298, 299) and apolipoprotein A-I levels (298) as well as increased levels of lipoprotein A (300) were observed among subjects with VaD. However, this association was not found in other studies (301, 302). Conflicting results were also noted in studies relating total cholesterol (303, 304), HDL-C (300, 305, 306) and LDL-C (303, 305) levels with Alzheimer's disease. A recent cohort study conducted by Reitz and colleagues (307) among 4,316 Medicare recipients, 65 years and older, residing in Northern Manhattan, NY, showed that there is a weak association between lipid levels and the risk of VaD. Similarly, the risk of AD was independent of both lipid levels and use of agents to lower them. Cardiovascular and metabolic risk factors such as hypertension and diabetes have been hypothesized to play a role in the pathogenesis of Alzheimer's disease (AD) as well as in development of vascular dementia (293, 308-310). The metabolic syndrome which is defined as the clustering of several conditions and disorders namely abdominal obesity, hypertriglyceridemia, low HDL, hypertension and hyperglycemia was recently studied by Yaffe and colleagues (144) as a risk factor for cognitive decline. A secondary objective of that study was to look for potential effect modification by inflammation. For that purpose, 1,616 elders were recruited and it was found that around two thirds of them screening positive on the metabolic syndrome. The main findings suggested that among high-functioning elders, those with metabolic syndrome exhibit an increased risk of developing cognitive impairment and decline over 4 years. This association remained after adjusting for demographic and lifestyle variables as well as chronic health conditions.

The increased rate of cognitive impairment was primarily observed in those elders who had high levels of serum markers of inflammation, suggesting that at least some of the increased risk associated with the metabolic syndrome is modified by inflammation.

An association between depressive symptoms and cognitive decline has been observed in selected cohorts of older people, but studies of defined populations have had conflicting results. A recent study conducted on 4,392 older people from a defined community in Chicago assessed global cognition at two points in time, around 5.3 years apart, based on four performance tests. Depressive symptoms were measured at baseline using a 10-item version of the CES-D scale. Results suggested that for each depressive symptom, the rate of cognitive decline increased by a mean of about 5%. This relationship did not change after control for presence of chronic illness, after exclusion of subjects who were cognitively impaired at baseline and was not modified by age, sex, race or education. Hence, it was suggested that depressive symptoms predict cognitive decline in old age (159). A number of other studies came to similar conclusions in the overall population of older adults (311-313), while other studies did not find an association (314, 315) and still others found the association only in those with baseline cognitive impairment (154) or relatively more education (316).

Specific families of drugs and medications have been studied in relation to cognitive functioning and these included: *psychotropic* drugs (e.g. anti-depressants, anxiolytics and anti-psychotic drugs), Non-Steroidal Anti-Inflammatory Drugs (*NSAIDS*), *statins* and *anti-hypertensives*. Psychotropic drugs are often hypothesized to induce cognitive decline in the elderly. A study by McShane and colleagues (317) investigated the contribution of neuroleptic drugs to cognitive decline in dementia among 71 subjects that were followed prospectively.

They found that the start of neuroleptic treatment coincided with more rapid cognitive decline: median rate of decline was 5 MMSE (interquartile range 8.5) points per year before treatment and 11 MMSE (interquartile range 12) points per year after treatment (P = 0.02). They concluded that neuroleptic drugs that are sometimes used to treat behavioural complications of dementia may worsen already poor cognitive function. However, in a recent study by Podewils and colleagues (318), findings failed to support the concept that tric-cyclic antidepressants (TCA) use is related to concurrent measurable cognitive deficits, and TCA use does not appear to significantly compromise memory over a substantial time span. A recent meta-analysis of NSAIDs and risk of dementia divided 25 reports into studies with prevalent dementia cases, studies with incident dementia cases, and studies where cognitive decline was used as the clinical endpoint. The pooled relative risks of the three groups of studies were 0.51 (95% CI: 0.37, 0.70), 0.79 (95% CI: 0.68, 0.92) and 1.23 (95% CI: 0.70, 2.31). The authors concluded, however, that most of the reported beneficial effects of NSAIDs may result from various forms of bias particularly recall, prescription, and publication biases (319). It was observed that lipid lowering drugs such as hydroxymethylglutaryl coenzyme A reductase inhibitors also known as *statins* may lower the risk of AD (320, 321) and VaD (321). A prospective randomized trial which was double-blind with treatment cross-over investigated the effects of six anti-hypertensive medications on short-term cognitive performance (162). The interventions consisted of six-week treatment periods of atenelol, metoprolol, hydrochlorothiazide, methyldopa, enalapril and verapamil, and 2-week placebo baseline and wash-out periods. In-depth neuropsychological assessments was conducted at baseline and after treatment. It was shown that irrespective of medication type, treatment reduced motor speed and slowed completion of two tests measuring peceptuo-motor speed and mental flexibility ($P<0.05$).

Manual dexterity declined somewhat with two medications (metoprolol and methyldopa). In contrast, anti-hypertensive agents had a favorable effect on working memory-related performance. A review article by Amenta and colleagues (322) presents evidence from previous studies that Ca^{2+} channel blockers and ACE inhibitors are more effective than diuretics and beta-blockers on cognitive domains of hypertension.

In summary, there are many risk factors that were studied in relation to cognitive impairment in general, and cognitive decline in particular. Epidemiologic studies have been conducted to look for the effects of socio-economic factors, mainly educational attainment, early childhood socio-economic position, and social disengagement, and assess the validity of alternative hypotheses regarding the presence of behavioral or health-related mediating factors. Genetic and hormonal factors were also the focus of many recent epidemiological studies as well as clinical trials. In general, Apo Eε4 allele was associated with increased risk of AD-type of dementia and cognitive decline. In terms of hormonal factors, most observational studies found either no effect or a protective effect of estrogen replacement therapy while a recent clinical trial found that exogenous estrogen has deleterious effects on cognitive functioning. Several behavioral risk factors have been studied in relation to cognitive performance. While many studies found weak or no association between smoking and cognitive decline, at least six other studies found a positive association whereby smoking increased the risk of decline. Alcohol was found in general to have a U-shaped association with the risk of decline, while caffeine was shown to increase perceptual speed and vigilance as well as memory and other more complex functions. Physical activity was shown to protect against cognitive decline as corroborated by a number of prospective studies. Nutritional factors that have been hypothesized to increase the risk of cognitive decline included BMI and excessive weight gain. However, few studies have been conducted to test this hypothesis.

Dietary and supplemental antioxidants were shown in some studies to reduce the risk of cognitive decline while in others they showed no appreciable effect. Other micronutrients including B-Vitamins and folate have been shown to be protective against decline as well.

Several co-morbid conditions have been associated with dementia and cognitive impairment, and these include stroke, cardiovascular disease, peripheral vascular disease, hypertension and diabetes as well as the general disease entity known as "metabolic syndrome". An association between depression and cognitive decline has been observed in cohorts of older people, although some results were inconclusive. Finally, specific families of drugs and medications have been studied in relation to cognitive functioning, and these included psychotropic drugs, NSAIDs, statins and anti-hypertensives.

Because of their potential effect on the outcome, many of these risk factors should be considered as potential confounders in the analysis after ensuring that they are not on the causal pathway between exposure and outcome.

B.3. Epidemiologic studies on health effects of ω-3 fatty acids

B.3.1. Cardiovascular, cerebrovascular and metabolic disease

Historically, the association between ω-3 fatty acids and cardiovascular disease was established following the observation that the Greenland Inuit had low mortality from coronary heart disease despite a diet that is rich in fat. In the 1970s, the Danish investigators Bang and Dyerberg proposed that this could be because of the high content of ω-3 fatty acid in the Inuit diet, which consisted largely of fish, seal and whale. In general, it has been suggested that direct consumption of long-chain ω-3 PUFA (mainly DHA and EPA) has a more potent impact in reduction of cardiovascular, cerebrovascular and metabolic disease risk than LNA intake alone via beneficial alterations in plasma membrane characteristics or activation of transcription factors.

In addition, high intake of LA which is prevalent in most Western countries tends to inhibit the efficient conversion of LNA into the longer chain ω -3 PUFAs (323). Fish consumption has been associated with a lower risk of coronary heart disease (CHD) in some but not all studies. A recent meta-analysis of nineteen observational studies that looked at this association indicated that fish consumption (vs. little or no fish consumption) was associated with fatal CHD with a pooled relative risk of 0.83 (95% CI: 0.76, 0.90; $p<0.005$). A similar result was obtained for total CHD with a relative risk of 0.86 (95% CI: 0.71, 0.92; $p<0.005$). It was found that this protective effect was more pronounced among women, when the pooled results were stratified by gender (324). Another meta-analysis of thirteen cohort studies which included 222,364 individuals with an average follow-up period of 11.8 years obtained a pooled multivariate RRs for CHD mortality of 0.89 (95% CI, 0.79, 1.01) for fish intake of 1-3 times per month, 0.85 (95% CI: 0.76, 0.96) for once per week, 0.77 (95% CI: 0.66, 0.89) for 2-4 times per week, and 0.62 (95% CI: 0.46, 0.82) for 5 or more times per week. Each 20 grams per day increase in fish intake was related to a 7% lower risk of CHD mortality (P for trend = 0.03) (325). A recent study using ARIC cohort data from the Minneapolis White community (n=3,591) suggested that a higher proportion of saturated fatty acids in the cholesteryl ester and phospholipid fractions of plasma was found among those who developed CHD after a mean follow-up of 10.7 years. However, no association was noted for ω -3 fatty acids, although an inverse association was found for arachidonic acid (an ω -6 long-chain fatty acid); (326).

Results from various observational studies on the relationship between fish consumption and stroke are inconsistent. A meta-analysis identified nine independent cohorts that provided a relative risk (RR) and corresponding 95% CI for total or any type of stroke in relation to fish consumption.

Pooled RR with their 95% CI were estimated to be 0.91 (95% CI: 0.79, 1.06) for individuals with fish intake 1 to 3 times per month, 0.87 (95% CI: 0.77, 0.98) for once per week, 0.82 (95% CI: 0.72, 0.94) for 2-4 times per week, and 0.69 (95% CI: 0.54, 0.88) for ≥5 times per week (P for trend =0.06). The results were more significant and in the direction of a protective effect for ischemic stroke but not so for hemorrhagic stroke (327). A recent cohort study of 4,775 elderly subjects (aged 65-98 years) who were free of known cerebrovascular disease at baseline and followed up for 12 years, suggested that while consumption of tuna, baked or broiled fish reduced the risk of ischemic stroke by up to 30%, consumption of fried fish/fish sandwich increased the risk by around 44%. Fish consumption in this study was not shown to be associated with hemorrhagic stroke (328). In terms of plasma levels of fatty acids, a nested prospective case-control study conducted among Japanese subjects aged 40 to 85 years suggested that a high serum level of linoleic acid is actually protective against total and ischemic stroke with respective odds ratios being 0.72 (95% CI: 0.59, 0.89) and 0.63 (95% CI: 0.46, 0.88) (329).

In addition to their effect on CHD and stroke, fatty acids were studied in relation to hypertension. A recent study by Zheng and colleagues (330) using the ARIC data from the Minneapolis center showed that the 6 - year incidence of hypertension was positively affected by the reduced levels of linoleic acid and the P/S ratio as well elevated levels of palmitic and arachidonic acids in the cholesteryl ester fraction of plasma. Similar findings were observed by Grimsgaard and colleagues (331) in a cross-sectional study of plasma phospholipid fatty acid concentrations and blood pressure, particularly in the adverse effect of saturated fatty acids and the beneficial effect of linoleic acid concentration. However, no specific effect of omega-3 fatty acids was reported in either study.

There has been evidence indicating that hemostatic profile is an important predictor of cardiovascular disease. However, studies were not able to establish which dietary factors are associated with a hypo or hypercoagulable profile. A study by Shahar and colleagues (332) using ARIC data found an inverse association between increased intake of ω -3 PUFAs and fibrinogen content in plasma as well as factor VIII, and vWF (for whites and blacks) and a positive association with protein C (for whites only). These findings suggested that a 1 serving per day increase in fish intake would lower fibrinogen by -2.9 mg/dL (-6.3, 0.5), factor VIII by -3.3% (-5.4, -1.3) and vWF by -2.7% (-5.2, -0.1) and would increase protein C by +0.07 microgram/mL (0.03, 0.11). Hence, it was concluded that increased intake of ω -3 PUFAs from fish could modify several coagulation factors and hence create an overall hypocoagulable profile. However, other studies have been controversial in their findings. While some had reproduced that protective effect of ω -3 PUFAs with respect to hypercoagulation (333-336), others failed to do so (337-339).

Prospective studies of human subjects report a protective effect of previous fish intake on the development of insulin resistance (340, 341). In addition, most intervention trials focusing on diabetic patients or patients with impaired glucose tolerance came to similar conclusions (342, 343), although some did not find any significant improvement in insulin sensitivity (344). Overall, animal studies consistently suggest a beneficial impact of LC ω -3 PUFA on insulin sensitivity, while evidence in human subjects is limited and largely inconclusive (323).

In addition, LC ω -3 PUFA have been investigated in relation to dyslipidemia and have been consistently found to reduce the level of triacylglycerides (TAG) in plasma among healthy (345) and hyperlipidaemic patients (346) upon their supplemention at a level of 3-4 grams/day. These effects were noted not only on fasting levels of TAG but also on postprandial levels among both normal (347) and hypertriacylglycerolaemic patients (348). Most reports indicate a favorable effect of fish oil on HDLs, although very high intake of fish oil may lower HDL concentrations.

The effects of fish oil on LDL metabolism represent the more controversial aspects of the ω -3 fatty acids effects. In fact, fish oil tended to increase the level of LDL particles as well as their average size. However, reduced LDL synthesis has been reported with large amount of fish oil. Moreover, ω -3 enrichment of the diet often renders LDLs susceptible to oxidation increasing the progression of atherogenesis. This fact indicates the need for antioxidant action such as that provided by α-tocopherol when large amounts of fish oils are to be consumed (349).

B.3.2. Cancer, depression, pulmonary function

There is increased evidence from animal and in vitro studies that ω -3 fatty acids, especially EPA and DHA, are able to inhibit carcinogenesis. Epidemiologic data on the association between fish consumption, as a surrogate marker for ω -3 fatty acid intake, and cancer risk are, however, somewhat less consistent. Most studies focused on breast and prostate cancer. According to a review by Larsson and colleagues (350), ecologic studies have shown that high per capita fish consumption is correlated with lower incidence of cancer in population. However, analytic case-control or cohort studies did not come to these conclusions in a consistent way. In fact, while some studies found an inverse association between fish consumption and cancer risk, most did not. Nevertheless, several molecular mechanisms for this putative association were proposed, including suppression of arachidonic acid-derived eicosanoid biosynthesis, influences on transcription factor activity, gene expression, and signal transduction pathways, alteration of estrogen metabolism, increased or decreased reactive oxygen species, and mechanisms involving insulin sensitivity and membrane fluidity. Although these mechanisms have been tested in vitro and in animal models, it is important to test their plausibility in humans and thus gain more insight as to the effects of ω -3 fatty acids on cancer risk.

Depression is yet another condition that has been associated with poor intake of ω -3 fatty acids. Hibbeln and his colleagues (351, 352) have proposed that ω -3 long-chain PUFA is an important factor underlying increased vulnerability to depression, as well as hostile and aggressive behavior. In part, this hypothesis was based on observing lower prevalence rates of depression in societies where high relative intake of ω -3 long-chain PUFA was maintained as compared to societies where there was a significant rise in the intake of ω -6 long PUFAs (mainly from vegetable oils) accompanied by a low intake of fish oils such as the United States. However, recent studies such as that conducted by Adams and colleagues (353) gave more specific evidence that severity of depression in moderately to severely depressed patients was highly correlated with blood measures of phospholipid fatty acid composition, specifically with high ratio of ω -6 long-chain PUFA : ω -3 long chain PUFA (e.g. AA:EPA); ($P<0.05$). Other studies have shown similar results with serum or erythrocyte levels of long chain fatty acids in relation to depression (354-356).

It has been hypothesized that through the attenuation of several components of inflammatory response, DHA and EPA are both able to prevent Chronic Obstructive Pulmonary Disease (COPD) that is often caused by smoking. Two studies using the ARIC data have shown that both dietary and plasma levels of ω -3 fatty acids, especially DHA, are able to prevent smoking-related COPD (332, 357). In the first study which measured dietary fatty acid exposure, the adjusted odds ratio of COPD and upper quartile of ω -3 fatty acid intake (vs. lower quartile) among ever-smokers was 0.59 with a 95% CI: 0.46-0.75. The logistic model used adjusted for age, sex, race, pack-years of cigarettes smoking, energy intake and educational level. Using similar definitions for COPD but using ω -3 fatty acid quartile distribution in plasma phospholipids, the second study restricted its study population to white middle-aged men and women from the ARIC Minneapolis center at visit 1 who were current smokers (n=2,349).

Similar results were obtained with respect to the odds ratio of quartiles of plasma DHA (vs. lower quartile) and COPD, with a clear dose-response relationship: 0.65, 0.51, 0.48 ($P<0.001$).

In summary, fish consumption as well as intake of ω -3 fatty acids have been associated with a wide array of health outcomes, which include reduced risk of coronary heart disease (CHD) and stroke as shown by a meta-analysis of a large number of observational studies, a favorable effect on HDL-C and Triacylglycerol (TAG), as well as reduced synthesis of LDL-C, increased insulin sensitivity, and a hypocoagulable profile.

Even through there was increased evidence from animal and in vitro studies that ω -3 fatty acids, especially EPA and DHA, are able to inhibit carcinogenesis, there was lack of consistent evidence particularly among cohort and case-control studies. Depression is yet another condition that has been associated with poor intake of ω -3 fatty acids. Finally, ω -3 fatty acids were shown to reduce the risk of chronic obstructive pulmonary disease (COPD).

B.4. Biomarkers of fat and fatty acids intake

B.4.1. Biomarkers and their time frame

Fat and its component fatty acids is a macronutrient the consumption of which has changed both in amount and type over time, particularly within Westernized countries. There are many reasons why it is difficult to assess intake of fat and fatty acids which include the hidden nature of many fats, variation of fatty acids contained in foods and feed as well as the degree to which respondents are sensitive to questions about fat intake in their diet. For these reasons, it is particularly desirable to make use of biomarkers. Technological and biological advances have made possible the quantification of fatty acids in various tissues and enhanced our appreciation of the differences between fatty acids of varying chain lengths and stereochemistry.

The main challenges that remain, however, are to determine which fraction of a biomarker reflects intake of fat and fatty acids, how to measure absolute vs. relative intake of fat and fatty acids; whether the biomarker actually reflects individual differences (e.g. genetic, disease, lifestyle factors, circulating apolipoprotein and hormonal milieu) that can influence deposition and mobilization of fat and to what extent endogenously produced fat is a contributor to the determined amount in various tissues (9). The association between dietary fat intake and coronary heart disease drew the attention towards trying to find a biochemical indicator for total fat intake. However, so far, there was no real success at finding such a biomarker particularly when linking fat intake to serum cholesterol and triglyceride fractions. In particular, the extent and shape of the relationship between total serum cholesterol and dietary fat has been discussed and based on metablic ward studies, Keys and Hegsted proposed equations that predict serum cholesterol from dietary intake of cholesterol, saturated and polyunsaturated fatty acids (358, 359). While Keys equation suggests that plasma cholesterol increases in proportion to the square root of dietary cholesterol (in mg/1000 kcal per day), the Hegsted equation proposed a linear relationship. Both equations seemed to perfom well over the usual range of cholesterol in developed countries. However, in low and high ranges, the Keys equation was shown to be superior in that respect (359). The equations are summarized as follows:

The Keys equation: $\Delta y = 1.35\ (2\Delta S - \Delta P) + 1.5\ \Delta Z$, where Δy = change in serum cholesterol (mg/dl); ΔS and ΔP = change in dietary intake of saturated and polyunsaturated fatty acids expressed as percentage of calories; and $\Delta Z = (x_1^{0.5} - x_2^{0.5})$, where x_1 and x_2 are the dietary cholesterols of the two diets being compared in mg/1,000 kcal.

The Hegsted equation: $\Delta y = 2.16\Delta S - 1.65\Delta P + 0.176\ \Delta C$, where ΔC is change in cholesterol intake of mg/1,000 kcal.

These equations were shown to perform well when comparing dietary interventions among groups. However, their use to calculate cholesterol intake among individuals from serum cholesterol resulted in severe misclassification. Data from many intervention and few observational studies support this assertion (360-362). In contrast to using Keys and Hegsted equations and serum cholesterol as a marker of fat intake, specific fatty acid levels in blood, cell membranes, and subcutaneous fats are more promising indicators of dietary fat intake, particularly with the advances in gas chromatography and HPLC that have been witnessed over the past few years that made it feasible to detect individual fatty acids and their isomers. It is reasonable to expect that the best indicators of fat intake are fatty acids that cannot be produced endogenously, namely the essential omega-3 (medium-chain derived from plants and long-chain derived from marine animals) and omega-6 fatty acids (mostly derived from plant oils), the trans-fatty acids (hydrogenated fats and ruminants), and odd-numbered and branched chain fatty acids (from dairy products). Linoleic acid (18:2 ω -6) is the principal dietary essential fatty acid which can be metabolized into longer chain fatty acids, mainly into arachidonic acid (20:4 ω -6). Similarly, linolenic acid (18:3 ω -3) gives rise to a series of longer chain omega-3 fatty acids including DHA and EPA (363). Although oleic acid (18:1n-9) is non-essential and can be produced endogenously, it has been shown to interact in a complex way with the metabolism of linoleic and linolenic acid. In fact, intake of any of these three fatty acids inhibits the elongation and unsaturation of the others (364).

It is important to keep in mind that markers of fatty acid intake are usually expressed as relative percentages of total fatty acids. Thus, an increase in dietary intake of one fatty acid, if incorporated into the substrate for analysis, results in a decrease in the relative amounts of the other fatty acids. This decrease should not be interpreted as a metabolic interaction with the exception of the one discussed above for linoleic, linolenic and oleic acids.

The substrates used to measure individual fatty acid relative amounts include erythrocytes, platelets, and adipose tissue, as well as several lipid subfractions found in plasma. These subfractions are cholesterol ester, phospholipid, and triglyceride fractions of serum or plasma or measurement can be made on free fatty acids. These fractions are separated usually by thin layer chromatography before proceeding to identification of individual fatty acids. It is still controversial how each of these substrates is related to dietary intake and what time frame of intake it usually mirrors. Nevertheless, several studies suggest that adipose tissue level of fatty acids provides the best measure for long-term intake and that other tissue fractions such as phospholipids in plasma, erythrocytes or platelets as well as plasma cholesteryl esters provide a biomarker for short to medium-term intake that is more responsive to changes in dietary patterns (363).

The ability to demonstrate a correlation with biochemical indicators is particularly valuable for nutrients that are essential and hence produced exogenously, namely vitamins and essential fatty acids. Several studies were able to prove the existence of a positive but weak to moderate correlation between various potential biomarkers of fatty acid intake and their level as assessed by different dietary assessment tools. These correlation coefficients were particularly elevated for long chain ω -3 fatty acids ranging between 0.18 and 0.74. These values were similar for linoleic (range: 0.22-0.77) and linolenic acid (range: 0.12-0.68) (365-379). While some of these studies controlled for extraneous factors such as age, sex, body mass index and smoking, others failed to do so. **Table 2.8** summarizes some of these results and shows which instruments were used for dietary assessment as well as the type of biomarker.

Table 2.8 Correlation between dietary and biomarker values of essential fatty acids: results from a systematic literature review

Source	Study Population	Dietary Assessment tool	Biomarker Type(s)	Fatty acid (Pearson Correlation)
(367)	40-75y (Men) (N=118)	two 7-day diet records	Adipose tissue	EPA (0.47)
(368)	40-64 years (both genders) (N=86)	two 7-d weighed-diet records	Adipose tissue	EPA (0.15 for men and 0.61 for women) DHA (0.47 for men and 0.57 for women)
(369)	Both genders (N=191)	104-item FFQ	Erythrocytes	Linoleic acid (0.44)
(371)	45-64 y; ARIC (both genders) (N=3,570)	Revised Willet 61-item FFQ	Plasma phospholipids Plasma Cholesteryl esters	Linoleic (0.22) Linolenic (0.15) EPA (0.20) DHA (0.42) Linoleic (0.28) Linolenic (0.21) EPA (0.23) DHA (0.42)
(380)	Both genders (N=24)	Multiple 7-d weighed food records	Adipose tissue	ω -3 PUFA vs. DHA in adipose tissue: r=0.58.
(372)	(Both genders) (N=363)	Quantitative FFQ (180-item)	Plasma phospholipids	EPA (0.51) DHA (0.49)
(373)	Middle aged Women (N=234)	Comprehensive FFQ	Plasma phospholipids	EPA (0.58) DHA (0.53)
(374)	Men (N=87)	Four 7-d weighed food records	Plasma phospholipids	EPA (0.75) *n-3* DPA (0.49) DHA (0.50)
(375)	39-61 years (Both genders) (N=858)	7 day diary (7DD) Self-administered FFQ	Plasma Cholesteryl esters	(1) 7 DD Linoleic acid: r=0.41-0.62 (men vs. women) (2) FFQ Linoleic acid: r=0.38-0.53 (men vs. women)

(Cont'd)

Source	Study Population	Dietary Assessment tool	Biomarker Type(s)	Fatty acid (Pearson Correlation)
(376)	Middle-aged (Both genders) (N=503)	135-item FFQ	Adipose tissue	ω *-3 fatty acids:* Linolenic (0.34) EPA (0.18) DHA (0.18) ω *-6 fatty acids:* Linoleic (0.58)
(377)	Seventh day Adventists (Bi-racial, both genders) (N=49 blacks, 72 whites)	200-item FFQ 8 24-hour recalls	Adipose tissue	*With 24-hour recalls*[4]: Linoleic (0.77,0.71) Linolenic (0.68, 0.62) *With FFQ*: Linoleic (0.61, 0.52) Linolenic (0.29, 0.49)
(378)	Overweight subjects (Both genders) (N=91)	Diet History Questionnaire	Erythrocytes	Six-month period: EPA: (0.66) DHA: (0.74) Total ω -3: (0.51)
(379)	23-63y (Both genders) (N=84)	Fat intake questionnaire (FIQ) Diet History Questionnaire (DHQ)	Adipose tissue	Linoleic FIQ: (0.58) DHQ: (0.49)

B.4.2. Lands model for fatty acid intake and biomarkers

While most studies assumed a linear relationship between dietary fatty acid intake and different biomarkers, the metabolic interactions of these fatty acids upon assimilation into different compartments dictates a different picture. Biochemical studies in the area of lipid research conducted by Lands and colleagues (381, 382) suggest that the mixture of 20-C and 22-C highly unsaturated fatty acids (HUFAs) that are maintained in phospholipids of human plasma is related to the dietary intake of 18:2(ω-6) and 18:3 (ω -3) (together named as ω-3 and ω-6 UFA: unsaturated fatty acids) by empirical hyperbolic equations in a manner very similar to the relationship reported for laboratory rats (383).

[4] Pearson correlation coefficients presented for black and whites, respectively.

Analytic results from volunteers ingesting self-selected diets showed an inter-individual variance for the proportion of (ω-6) eicosanoid precursors in the fatty acids of plasma phospholipids of about 5%, but the variance among multiple samples taken from the same individual throughout the day was less (about 3%), which was closer to the analytic procedure conducted under experimental conditions (about 1%). The authors concluded that the reproducibility of the results makes it likely that analysis of fatty acid composition of plasma lipids from individuals will prove useful in estimating diet-related tendency for severe thrombotic, arthritic or other disorders that are mediated by (ω -6) eicosanoids.

The empirical equations relating (ω-6) and (ω-3) HUFAs as % of total HUFAs in plasma phospholipids to dietary intake of 18:2(ω-6) and 18:3(ω-3) was hyperbolic in nature and was successful at reflecting the general metabolic selectivities that maintain fatty acid composition in plasma phospholipids. Additional constants and terms were included in the equations to account for the effects of 20-C and 22-C highly unsaturated (ω-3 and ω-6) fatty acids in the diet. Differential competition between the long chain and short ω -3 fatty acids to decrease the ability of 18:2(ω-6) to maintain 20:4(ω-6) in plasma phospholipids was reflected by a set of constants that were incorporated in each of these hyperbolic equations. These constants with their formal definition are presentd in **Table 2.9**. The sets of hyperbolic equations can be used in reverse to estimate dietary intake of the (ω-3) and (ω-6) fatty acids by using the composition of the fatty acids that had been maintained in plasma phospholipids. The specific method to be used will be discussed later (Chapter IV) when we look at the validation sub-study methodology. In general, these equations were derived based on the following competitive hyperbolic relationship commonly used to describe saturable rate-limiting biochemical processes:

$$\text{Response} = \frac{V_{max}}{1+\frac{K_m}{en\%S}(1+\frac{en\%I}{K_i})}$$

Where V_{max} in this case is 100 (since the response is a percentage), K_m and K_i are constants that correspond to the type of fatty acid in question. Further modification of this general relationship in order to best fit the data resulted in the equations that are presented in Table 4.7 of Chapter IV. **Table 2.9** lists the different constants, their definitions and empirically determined values.

Table 2.9 Constants included in the hyperbolic empirical equations: Definitions and values (381, 382)

Constant	Definition	Value
C_3	Standard effective concentration of 18:3 (ω -3) as a percentage of total caloric intake	0.0400
C_6	Standard effective concentration of 18:2 (ω -6) as a percentage of total caloric intake	0.0600
C_o	Constant for the effect of other dietary fatty acids (non-essential).	5.0
K_S	Constant for shape fitting	0.175
PC_3	Standard effective concentration of 18:3 (ω -3) as a percentage of total caloric intake	0.0555
PC_6	Standard effective concentration of 18:2 (ω -6) as a percentage of total caloric intake	0.0441
HI_3	Competitive inhibition by the dietary ω-3 HUFA in elongation and desaturation of the (ω -3) and (ω -6) UFA.	0.005
HI_6	Competitive inhibition by the dietary ω-6 HUFA in elongation and desaturation of the (ω -3) and (ω -6) UFA.	0.040
HC_3	Efficiency of direct esterification of dietary (ω -3) HUFA.	3.0
HC_6	Efficiency of direct esterification of dietary (ω -6) HUFA.	0.70

In summary, while most studies assumed a linear relationship between dietary fatty acid intake and different biomarkers, the metabolic interactions of these fatty acids upon assimilation into different compartments dictates a different picture. I propose to use a set of empirical equations and biomarkers derived from plasma phospholipid levels of specific highly unsaturated fatty acids to estimate the true level of fatty acid intake in the diet. Other instrumental biomarkers include the corresponding level of fatty acids in phospholipids and cholesteryl ester fractions of plasma.

C. Critical Review of the literature

C.1. ω-3 fatty acids and cognitive functioning

Several epidemiological studies have shown that the biochemical composition of blood components in terms of fatty acids differs significantly between subjects with normal cognitive functioning and patients with some form of cognitive impairment. A study by Conquer and colleagues (384) investigated the fatty acid plasma composition among patients with Alzheimer's disease, other types of dementia and cognitive impairment. Findings suggested that patients with either one of these conditions had lower plasma phosphatidyl choline (PC) level of ω-3 fatty acids which include EPA and DHA as well as a lower ratio of ω -3/ ω -6 fatty acids when compared to normal controls. Similar findings were shown for the other two phospholipids fractions that were analyzed which were phosphatidylethanolamine (PE) and lysophosphatidylcholine (lysoPC). The authors concluded that a decreased plasma level of ω-3 fatty acids, and in particular DHA, is associated with Alzheimer's disease as well as other forms of cognitive impairment. Another case-control study conducted by Tully and colleagues (385) used an established marker of ω-3 polyunsaturated fatty acid intake (serum cholesteryl ester-fatty acid composition) to determine ω-3 PUFA status in patients with Alzheimer's disease as assessed by both Mini-Mental State Examination (MMSE) score <24/30 and clinical dementia rating (CDR) and among controls who had normal cognitive functioning as assessed by MMSE ≥24/30. Results showed that patients with Alzheimer's disease had significantly lower levels of serum cholesteryl ester-eicosapentaenoic acid (EPA) as compared to the controls. A third recent study adopted a nested case-control design within the Epidemiology of Vascular Aging or EVA cohort. Its main aim was to assess fatty acid composition of erythrocyte membranes as a risk factor for cognitive decline among 246 men and women aged 63-74 years within the 4-year follow-up which was conducted in France.

The study found that total ω-6 polyunsaturated fatty acids were associated with a greater risk of cognitive decline with an odds ratio of 1.59 (95% CI: 1.04, 2.44). Conversely, a higher proportion of total ω -3 fatty acids were associated with a lower risk of cognitive decline, with an odds ratio of 0.59 (95% CI: 0.38, 0.93). Hence, overall there was an inverse association between cognitive decline and the ratio of ω-3/ ω-6 fatty acids in erythrocytes (386). While the majority of these studies showed a preventive effect of plasma and erythrocyte ω -3 fatty acids on cognition among older adults, others found either no effect or the reverse effect. In fact, a case-control study – the Canadian Study of Health and Ageing – showed that the mean relative plasma concentration of ω-3 fatty acids as well as total polyunsaturated fatty acids was higher among subjects aged 65 years or more with cognitive impairment or dementia after controlling for age, sex, education, smoking, alcohol intake, body mass index, history of cardiovascular disease, and apolipoprotein E ε4 genotype (387).

Epidemiological studies involving dietary assessment of ω-3 fatty acids had suggestive but slightly controversial results. One study by Morris and colleagues (273) used cohort data on 815 subjects who were initially unaffected by Alzheimer's disease and whose ages ranged between 65 and 94 years, while mean follow-up period was 2.3 years. Using standardized criteria, the incidence of Alzheimer's disease was compared across ω-3 fatty acid consumption groups, with those eating fish once per week compared to those who rarely or never eat fish having considerably lower incidence (RR=0.4; 95% CI: 0.2, 0.9). Total ω-3 fatty acid consumption was also associated with a reduced risk of Alzheimer's disease even after controlling for intake of other dietary fats, vitamin E and for cardiovascular conditions.

Findings from the Zutphen Elderly Study indicated that high linoleic acid intake was associated with cognitive impairment, even after controlling for age, education, cigarette smoking, alcohol consumption and energy intake (OR: 1.76, 95% CI: 1.04-3.01, comparing highest to lowest tertile). However, with regard to ω-3 fatty acids, there was no distinctive association. Nevertheless, total fish consumption was suggestive of a protective effect, even though it did not reach significance. Cognitive functioning and decline over a period of three years in this study were assessed among a cohort of 476 men in the age range 69 to 89 years, using MMSE (137). Another larger cohort study – The Rotterdam Study – recruited 5,395 subjects between the years 1990 and 1993 who had normal cognition, were non-institutionalized, and assessed their complete dietary intake with a semi-quantitative food-frequency questionnaire. Re-examination for assessment of incident dementia was carried out in 1994 and 1997 to 1999. After a mean follow-up period of 6.0 years, 197 subjects developed dementia with 146 developing Alzheimer's disease. The conclusions however were that high intake of total, saturated, trans fat, cholesterol and low intake of MUFA, PUFA, ω-6 PUFA and ω -3 PUFA were not associated with increased risk of dementia or its subtypes (141). A recent study by Kalmijn and colleagues (388) used cross-sectional data of 1,613 subjects ranging in age between 45 and 70 years to examine the association between fatty acid and fish intake with cognitive function. The authors found that the risk of cognitive impairment was reduced with increased consumption of fatty fish and marine ω-3 PUFA. In fact, per Standard Deviation (SD) increased intake, th(389)e odds ratios were estimated to be 0.81 (95% CI: 0.66, 1.00) and 0.72 (95% CI: 0.57, 0.90).

Some limitations of the studies described above include measurement error in case of dietary fatty acid assessment, cross-sectional design in some (388), small sample size in others (385), and a short follow-up period (385, 390).

Our present study will attempt to remedy for all these limitations, by correcting for measurement error through advanced statistical techniques and use of a large cohort with a follow-up period of 6 years.

C.2. Hypertension and cognitive functioning

Previous studies suggest the possibility of a relationship between elevated blood pressure in middle age and later cognitive decline. However, these studies do not all yield the same results and thus do not permit a final statement on this association. These differences seem to arise mostly from methodological and sampling variations between studies. However, in general, the majority of studies indicated that cognitive function tends to be poorer and decline to be faster with increased systolic or diastolic pressure or both. This finding applied only to men in certain instances and among subgroups with co-existing health conditions, while it was generalizable to both men and women in the population in other studies. In addition, this positive association was seen both among the elderly and middle-aged adults. An earlier cohort study conducted by Launer and colleagues (287) on 3,735 surviving Japanese-American middle-aged men from the Honolulu Heart Program (baseline, 1965-1968) showed that the risk of intermediate and poor cognitive function increased progressively with increasing level of midlife SBP category (P for trend <0.03 and 0.01, respectively) and remained statistically significant even after adjustment for age, education, prevalent stroke, coronary heart disease and sub-clinical atherosclerosis (RR for poor cognitive function and 10mm Hg increase in SBP was 1.05 with a 95%CI: 1.00-1.12). Cognitive function was assessed using the Cognitive Abilities Screening Instrument (CASI) at the fourth examination which took place within the period of 1992 through 1993. The effect of DBP on cognitive function was not statistically significant.

Another study by Kilander and colleagues (391) conducted among 999 seventy-year old men in Uppsala, Sweden aimed at analyzing the impact of hypertension, circadian blood pressure (BP) profile, and disturbed glucose metabolism at the age of 50 on cognitive function over a period of 20 years. Cognitive function was assessed using the Mini-Mental State Examination (MMSE) and the Trail-Making test. High diastolic BP at baseline predicted later impaired cognitive performance, even after excluding men with a previous stroke. This relationship was strongest among untreated men.

Swan and colleagues (392) conducted a longitudinal study over a period of 25 to 30 years on older adults and concluded that people who maintain elevated SBP throughout their adult lives are at increased risk for reduced verbal learning and memory function. However, a recent study by Glynn and colleagues (393) conducted among the Established Populations for Epidemiologic Studies of the Elderly (EPESE) cohort of East Boston aged 65 to 102 years at baseline suggested that there is no linear association between BP and cognitive decline but rather a U-shaped one. In this study, BP was measured both at baseline and 9 years prior to baseline among subjects who participated in another cohort (HDFP) and adjustment was made for age, sex and education.

A more recent study by Harrington and colleagues (394) suggested that hypertension in older subjects is associated with impaired cognition in a broad range of areas, such as reaction time, memory scanning, immediate word recognition and spatial memory. This association persisted in the absence of clinically evident target organ damage. Elias and colleagues (47) made use of the Framingham Heart Study to determine the independent effects of obesity and hypertension on cognitive functioning. Using a prospective design, around 1,423 participants were classified by presence or absence of obesity and hypertension based on data collected over an 18-year surveillance period.

All subjects were free from dementia, stroke, and clinically diagnosed cardiovascular disease up to the time of cognitive testing. Statistical models were adjusted for other known risk factors, such as age, education, occupation, cigarette smoking, alcohol consumption, total cholesterol, and a diagnosis of type II diabetes. Cognitive testing was done again 4-6 years later. Findings showed that adverse effects of obesity and hypertension on cognitive performance were observed for men only. Obese and hypertensive men performed more poorly than men classified as either obese or hypertensive, and the best performance was observed in non-obese, normotensive men.

Using the Atherosclerosis Risk in Communities (ARIC) data, several studies have looked at risk factors of cognitive impairment. Two of these looked specifically at hypertension in addition to other cardiovascular risk factors. The first one by Knopman and colleagues (150) performed a social neuropsychological assessment to detect vascular risk factors for cognitive decline in the ARIC cohort. The cognitive assessment was conducted on two occasions separated by a period of 6 years among adults aged 47 to 70 years at baseline. The three cognitive tests administered were the following: (1) Delayed Word Recall (DWRT) test, a 10-word delayed free recall task in which the learning phase included sentence generation with the study words; (2) Digit Symbol Subset (DSST) of the Wechsler Adult Intelligence Scale-Revised (WAIS-R); and (3) Word fluency (WFT) test using letters F, A, and S. The main findings of the study is that the presence of diabetes at baseline was associated with greater decline in scores on both the DSST and WFT ($p<0.05$). All other cardiovascular risk factors considered did not affect the cognitive scores significantly. Another study by Alves de Moraes and colleagues (44) made use of a similar methodology to assess cognitive decline over a period of 6 years, but focused on temporal changes in blood pressure as the main exposure. The results showed that older subjects with uncontrolled hypertension had a significantly larger mean DSST/WAIS-R score decline than normotensive subjects.

However, Stewart and colleagues (395) found no proof of an association between raised blood pressure (SBP>160 mmHg. and DBP>95 mmHg) and a composite score of cognitive decline computed using factor analysis and taking the lower quintile referring as the index category (OR: 1.46; 95% CI: 0.65-3.25). Finally, recent research on this association was able to uncover non-linear associations between blood pressure change over time and cognitive change through that same period of time using mixed regression models (174).

D. Synopsis

The literature presented above suggests that there is a biological evidence for an interaction between hypertension and ω-3 fatty acid status and intake in the diet in relation to cognitive performance. However, this interaction has not been translated to date into an epidemiologic investigation. It is therefore important to test this hypothesis at the population level and arrive at a public health policy related to the combined beneficial effects of preventing elevated blood pressure and increased intake of ω-3 fatty acids in the diet. **Table 2.10** describes the relevant literature presented in more detail. **Figure 2.2** presents the relationships between our main outcome of interest and selected risk factors as suggested by the literature. This directed acyclic graph will be instrumental in model selection as will be discussed in more detail in Chapter IV.

Table 2.10 Systematic review of the independent effects of ω-3 fatty acids and hypertension on cognitive impairment or decline

Author(s) (year) Study	Age (gender)	Design	Number of participants	Case Definition	Exposure definition	Follow-up time	Main findings
I. ω-3 fatty acids							
Kalmijn *et al.*, 1997 *Zutphen study* *Source*: (137)	69-89 (Men)	Cohort	939: impairment 342: decline	Cognitive impairment at baseline and decline over 3 years: MMSE, using <=25 as cutoff point for impairment	High vs. low consumption of fish[5]	~3 years	**OR (Impairment)[6]** 0.63 95% CI: 0.33-1.21 **OR (Decline)[7]** 0.45 95% CI: 0.17-1.16
Conquer *et al.* (2000)[8] *Source*: (384)	65+ (Both)	Case-control	N=19 (**AD**) N=10 (**OD**) N=36 (**CIND**) N=19 (**N**)	Incident AD, OD, CIND	Plasma phospholipid content of fatty acids (as % of total fatty acids)	n/a	Lower ω-3/ ω -6 ratio; total ω -3 and DHA among **AD**, **OD** or **CIND** as compared to **N** (P<0.05).
Engelhart *et al.*. (2002a) *Rotterdam Study* *Source:* (141)	55+ (Both)	Cohort	5,359	Incident dementias: MMSE, followed by CAMDEX, followed by Neurologic/neuropsychiatric examination + MRI (if possible)	ω -3 PUFAs[9]	~ 7 years	**IRR (total dementias)[10]** 1.07 95% CI: 0.94,1.22

[5] Cross-check diet history method, adapted to the Dutch population;

[6] Controlling for: Age Education Alcohol Smoking Total energy intake

[7] Controlling for: Age Education Alcohol Smoking Total energy intake

[8] AD: Alzheimer's Disease using NINCDS-ADRDA criteria; OD: Other dementias than AD; CIND: Cognitive Impairment, no dementia: Did not meet criteria for dementia according to DSM-IV but scored 1 SD lower than their norm for their age group in several neuropsychological tests (e.g. Digit symbol, visual and verbal memory, praxis etc.). ; N: Normal controls.

[9] Semi-quantitative FFQ, extensive, validated for the Dutch population; Checklist of food items and supplements added to the FFQ.

[10] Controlling for: Age, gender, education, total energy intake and vitamin E intake.

Author(s) (year) Study	Age (gender)	Design	Number of participants	Case Definition	Exposure definition	Follow-up time	Main findings
Tully *et al.* (2003) *Source*: (385)	49-92 (Both)	Case-control	148 cases 45 controls	Incident AD: Clinical Dementia Rating (CDR) and MMSE	Cholesteryl-ester ω -3 PUFAs	n/a	**DHA (mean):** Cases: 0.44 Controls: 1.07 P<0.05 **EPA(mean):** Cases: 1.05 Controls: 1.55 P<0.05
Heude *et al.* (2003) *EVA study* *Source:* (386)	63-74 (Both)	Cohort	246	Cognitive decline: MMSE decline: 2-points decline or more (moderate cognitive decline)	Erythrocyte membrane content of fatty acids	~4 years	**OR (DHA)[11]:** 0.60 95% CI: 0.39, 0.93 **OR(EPA):** 0.57 95% CI: 0.31, 1.04
Laurin *et al.* (2003) *Canadian Study of Health and Ageing* *Source:* (387)	65+ (Both)	Case-control	?	Incident cognitive decline cases and cases of dementia	Plasma phospholipid concentration of ω -3 fatty acids	n/a	Cases have higher DHA and EPA concentrations than controls (P<0.05)[12]
Morris *et al.* (2003) *Source*: (389)	65-94 (Both)	Cohort	729	Incident AD Diagnostic tests: team of neurologist, nurse practitioner, phlebotomist, and a neuropsychological technician.	Total ω -3 PUFA: upper vs. lowest quintile[13]	~ 4 years	**OR (total ω-3 PUFAs: upper vs. lowest quintile)[14]:** 0.4 95% CI: 0.1, 0.9
Kalmijn *et al.* (2004) *Source*: (388)	45-70 (Both)	Cross-sectional	1,613	Cognitive impairment: Memory, psychomotor. Speed, cognitive flexibility, and overall cognition.	SD increase in total ω -3 fatty acids[15]	n/a	**OR: Overall cognitive impairment for each SD increase in ω -3 fatty acids[16]:** 0.84 95% CI: 0.66-1.00

[11] Controlling for: Age, gender, education and baseline MMSE

[12] Controlling for: Age, sex, education, smoking, alcohol, body mass index, history of cardiovascular disease, ApoE-ε4 genotype.

[13] Harvard self-administered FFQ (139 food items)

[14] Controlling for: Age, gender, race, education, APOE-ε4

[15] Dutch EPID Food Frequency Questionnaire (178 food items)

[16] Controlling for: Age, sex, education, alcohol consumption, smoking and total energy intake.

Author(s) (year) Study	Age (gender)	Design	Number of participants	Case Definition	Exposure definition	Follow-up time	Main findings
II. Hypertension							
Launer *et al.* (1995) *The Honoloulu-Asia Aging Study* *Source*: (287)	Mid-aged (Men)	Cohort	3,735	Cognitive impairment: Intermediate and poor cognitive performance at fourth examination: Cognitive Abilities Screening Instrument (CASI): 92-100: Normal <92-82: Intermediate <82: Poor	Midlife SBP and DBP	~27 years	**RR for 10 mmHg increase in SBP and poor cognitive function[17]:** 1.05 95% CI: 1.00-1.12.
Kilander *et al.* (1998) *Uppsala Sweden study* *Source*: (391)	70 (Men)	Cohort	999	Cognitive impairment: Low cognitive performance on MMSE & Trail making test.	DBP (mm Hg.) 24 hours prior to cognitive testing	~20 years	**Mean DBP for Men untreated with anti-hypertensives[18]:** Normal: 75 mm Hg. Low: 78 mm Hg. P<0.0001 **Mean DPB for men treated with anti-hypertensives:** P>0.05.
Swan *et al.* (1998) *Western Collaborative Group Study* *Source*: (392)	60-86 (Men)	Cohort	717	Cognitive performance factor scores using several screening tools[19]	Change in SBP over a 30 year period. NBP SBP trackers SBP decreasers	~30 years	Referent: NBP[20] SBP trackers perform worse on Verbal memory and Verbal Fluency. SBP decreasers perform worse on Psychomotor Speed

[17] Controlling for: Age, education, prevalent stroke, coronary heart disease, sub-clinical atherosclerosis.

[18] Controlling for: Age, education and occupation.

[19] (1) Iowa Screening Battery for Mental Decline; (2) Mini-Mental State Examination; (3) Wechsler Adult Intelligence Scale-Revised; (4) Color Trail Making Test; (5) Color-Word Interference Test.

[20] Controlling for: Age, sex, education, depression, clinically defined stroke and use of anti-hypertensive medication.

Author(s) (year) Study	Age (gender)	Design	Number of participants	Case Definition	Exposure definition	Follow-up time	Main findings
Glynn *et al.* (1999) *EPESE study* *Source*: (393)	65-102 (Both)	Cohort	2,068	Rate of change in cognition measured by SPMSQ and EBMT	Current and remote SBP and DBP at and prior to baseline (9 years apart)	3 and 6 years	Difference in rate of change in cognition[21]: No statistically significant associations.
Harrington *et al.* (2000) *Source*: (394)	70-89 (Both)	Cross-sectional	223	Cognitive scores in several domains of the Cognitive Drug Research Computerized Assessment Battery.	Hypertensives: >160/90 mmHg. Normotensives: <150/90 mm Hg.	n/a	Difference in mean outcomes by hypertension status: Statistically significant for many domains including delayed word recognition, reaction time etc.
Knopman et al. (2001) *ARIC study* *Source*: (150)	47-70 (Both)	Cohort	10,963	Cognitive decline: Scores on Delayed Word Recall, Digit-Symbol Substitution test and Word Fluency test.	Hypertensive as measured at vist 2 (>140/>90 mm Hg.) or taking anti-hypertensives.	6 years	**Adjusted mean difference in scores of outcome between visits 2 and 4[22]:** No statistically significant association.
Alves de Moraes (2002) *ARIC study* *Source*: (44)	48-67 (Both)	Cohort	8,058	Cognitive decline: Scores on Delayed Word Recall, Digit-Sym bol Substitution test and Word Fluency test.	Combination of self-report of history of hypertension, or use of anti-hypertensive medication + measured SBP and DBP (>140/>90 mm Hg.)	6 years	**Adjusted percent change between visit 2 and visit 4[23]:** (V4-V2)/V2 -2.3% for DSST among *normal blood pressure* group. -7.5% for *uncontrolled hypertension* group. P<0.05

[21] Controlling for: Age, Age-squared, Age-cubed, sex, education and period.

[22] Controlling for: Gender, age, race-center, educational level, and use of CNS medications at visit 2.

[23] Controlling for: Gender, age, education, race-center, diabetes.

Author(s) (year) Study	Age (gender)	Design	Number of participants	Case Definition	Exposure definition	Follow-up time	Main findings
Elias *et al.* (2003) *Framingham Study* *Source*: (47)	55-88 (Both)	Cohort	2,123	Cognitive functioning Kaplan- Albert Neuropsychological Test Battery	Mean SBP and DBP computed over the surveillance period. Cutoff point: 140/90 mm Hg.	4-6 years	**Adjusted mean scores on cognitive test domains, comparing hypertensives to normotensives[24]** : Statistically significant only for men.
Stewart *et al.* (2003) *Source*: (395)	55-75 (Both)	Cohort	207	Cognitive decline Tests of verbal memory (immediate and delayed), orientation and attention. MMSE & CERAD Composite score of cognitive decline: Lowest quintile vs. other quintiles	Self-reported hypertension + measured blood pressure on examination.	3 years	**OR (Raised blood pressure vs. cognitive decline)** 1.46 (0.65-3.25).
Waldstein *et al.* (2005) *Baltimore Longitudinal Study of Aging* *Source*: (174)	50+ (Both)	Cohort	847	Cognitive functioning. Scores on Digit forward/backward (WAIS-R); California Verbal learning test; Benton Visual Retention test; Trail-Making test (Parts A and B); Letter fluency; Category fluency; Boston Naming Test.	Two resting blood pressure measurements taken at least 90 min. before breakfast. Measurements averaged.	Repeated measures 1-7 visits over 11 years.	**Mixed effect models[25]** of blood pressure and cognitive test scores, allowing for non-linear relationships: Cross-sectional and longitudinal relations of BP and cognitive function are predominantly non-linear and moderated by age, education and anti-hypertensive medication.

[24] Controlling for: Age, education, occupation, cigarettes/day, alcohol consumption, total cholesterol and type II diabetes.

[25] Controlling for: Age, education, alcohol use, smoking, use of anti-hypertensive medications, depressive symptomatology.

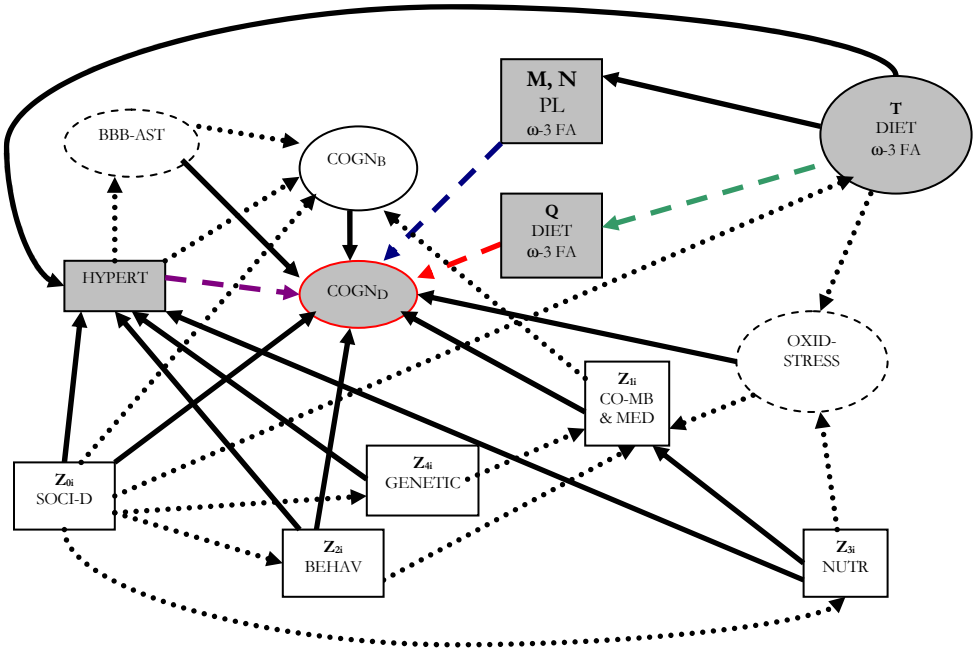


Figure 2.2 Directed Acyclic graph of association between ω-3 FA, hypertension and cognitive decline [26,27]

[26] Squared boxes represent measured variables; solid round boxes represent latent variables measured through a set of other variables; dashed round boxes represent unmeasured latent variables; Solid arrows represent causal associations for which the outcome is either our main outcome of interest, the main exposures or the potential effect modifier; Dashed arrows represent causal associations that are of most interest to the current analysis; and dotted arrows represent all other causal associations. Shaded boxes contain the variables of highest interest in the analysis.

[27] Score values for baseline cognitive functioning ($COGN_B$) and cognitive decline ($COGN_D$) will be determined for each domain as well as globally through a principal components analysis combining DWRT (delayed word recall), DSST (Digit symbol substitution) and WFT (Word Fluency) test scores. BBB-AST: blood-brain barrier/astrocytic dysfunction; OXID-STRESS: Oxidative stress; **Q** Diet ω-3 FA: Estimate of dietary ω-3 fatty as obtained from the Food-frequency questionnaire; **T** Diet ω-3 FA: True intake of dietary ω-3 FA; **M, N** PL ω-3 FA: Markers for level of ω-3 FA in phospholipids and cholesteryl ester fractions of plasma; HYPERT: Hypertension status; SOCI-D: age, gender, ethnicity and education; CO-MB: Stroke or TIAs, Depression, Type II Diabetes, Dyslipidemia, Poor pulmonary function, hypercoagulable profile and inflammation; use of psychotropic, anti-inflammatory drugs and statins; BEHAV: Smoking, alcohol and caffeine consumption, physical activity; NUTR: Body Mass Index (BMI), Antioxidant intake, folate and Vitamins B_6 and B_{12} GENETIC: ApoEε4 genotype.

C h a p t e r 3

STATEMENT OF SPECIFIC AIMS

A. Study Questions & Specific aims

The present study assessed the relationship between dietary as well as plasma ω-3 fatty acid and cognitive decline among older adults participating in the Atherosclerosis Risk in Communities (ARIC) study and look for an interaction with elevated blood pressure. ARIC is a cohort study of around 16,000 men and women aged 45 years or more at baseline who were selected from four US communities and followed up since 1987 at approximately one year intervals, and for whom clinical examination and specimen collection has been conducted so far at four points in time. The four visits that were completed longitudinally were: visit 1(1987-89), visit 2(1990-92), visit 3 (1994-95), and visit 4 (1996-98). The specific study questions are:

1. Is dietary essential fatty acid as assessed by the food frequency questionnaire a valid measure of intake and if so, what is the degree of measurement error in the instrument?

2. Is low *dietary* consumption of ω-3 fatty acids at baseline (i.e. visit 1) related to incidence of cognitive decline between visits 2 and 4 among older adults aged 50+ years at baseline?

3. Is low *plasma* ω-3 fatty acids at baseline related to cognitive decline between visits 2 and 4 among older adults aged 50+ years at baseline?

4. Do these two risk factors interact with hypertension between visits 2 and 4 to increase the risk of cognitive decline? In other words, is hypertension an effect modifier in the relationships that are investigated in questions 1. and 2.?

5. Does plasma ω-3 fatty acid interact with ApoE ε4 allele in increasing the risk of cognitive decline?

6. Is there a similar interaction with other oxidative-stress inducing conditions?

B. Hypotheses

AIM1:

Although this aim had no specific hypothesis, it focused on finding the degree of measurement error incurred by using dietary intake assessed with a semi-quantitative questionnaire and devised a method to correct for this error in future studies using the same dietary assessment tool on similar populations. This aim pertains to Question 1 above.

AIM 2:

This is the central aim of this work. The main hypothesis to be tested is that low ω-3 fatty acid status is associated with cognitive decline among older adults and a secondary hypothesis is that hypertension has a synergistic effect in that association. In other words, hypertension interacts with low ω-3 fatty acid statuses in a super-multiplicative way to increase the risk of cognitive decline. These hypotheses are operationalized as follows:

Question 2: Is low *dietary* consumption of ω-3 fatty acids at baseline (i.e. visit 1) related to incidence of cognitive decline among older adults between visits 2 and 4?

H_{21}: The dietary intake of medium-chain ω-3 fatty acid (mainly LNA) as percentage of total energy intake is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, other dietary fatty acids and potential confounders.

H_{22}: The dietary intake of long-chain ω-3 fatty acid (DHA+EPA+ω-3DPA) as percentage of total energy intake is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, other dietary fatty acids and potential confounders.

H_{23}: The dietary intake of long-chain and medium ω-3 fatty acid (or total ω-3 fatty acid) as percentage of total energy intake is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, other dietary fatty acids and potential confounders.

H_{24}: The ratio of long chain ω-3 fatty acids (DHA+EPA+ω-3DPA) to long chain ω-6 fatty acids (mainly AA) in the diet is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, other dietary fatty acids and potential confounders.

H_{25}: The ratio of total ω-3 fatty acids to total ω-6 fatty acids in the diet is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, other dietary fatty acids and potential confounders.

H_{26}: The ratio of medium chain ω-3 fatty acid (mainly LNA) to medium chain ω-6 fatty acid (mainly LA) in the diet is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, other dietary fatty acids and potential confounders.

Question 3**:** Is low *plasma* ω-3 fatty acids at baseline related to cognitive decline among older adults between visits 2 and 4?

H_{31}: Percent long-chain ω-3 fatty acid in plasma cholesteryl ester fraction (mainly DHA+EPA) is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, other fatty acid types in that fraction and other potential confounders.

H_{32}: The ratio of long-chain ω-3 to long chain ω-6 fatty acids in plasma cholesteryl ester fraction is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, medium chain ω-3 and ω-6 fatty acids in that fraction and other potential confounders.

H_{33}: Percent long-chain ω-3 fatty acid in plasma phospholipids fraction (mainly DHA+EPA) is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, other types of fatty acids in that fraction and other potential confounders.

H_{34}: The ratio of long-chain ω-3 to long chain ω-6 fatty acids in plasma phospholipids fraction is inversely related to the risk of cognitive decline, after controlling for baseline cognitive functioning, medium chain ω-3 and ω-6 fatty acids in that fraction and other potential confounders.

Question 4: Do these two risk factors interact with hypertension between visits 2 and 4 to increase the risk of cognitive decline? In other words, is hypertension an effect modifier in the relationships that are investigated in questions 1. and 2.?

H_{4i}: Low ω-3 fatty acid status as measured in hypotheses H_{11} through H_{24} is positively related to the risk of cognitive decline after controlling for baseline cognitive functioning, other dietary fatty acids and potential confounders and this association is stronger among hypertensive individuals.

AIM 3:

ω-3 fatty acids have been historically associated with reduced risk of cardiovascular disease including stroke (327) and coronary heart disease (324, 325). They were also linked with improved insulin sensitivity (341), reduced risk of dyslipidemia (346), a hypocoagulable profile (332), improved pulmonary function (357) and reduced risk of major depression (352) among other health benefits. Because many of these conditions were related to increased oxidative stress (52, 396-400) which in turn causes neuronal loss and cognitive impairment among older adults (401), it is essential to unveil any putative interaction between these conditions and the ability of this class of fatty acids to fulfill their beneficial effects.

An additional genetic factor -- having an ApoE ε4 allele -- has been consistently associated with increased risk of cognitive decline (213, 216) and is now one of the main biomarkers for fast progression into Alzheimer's disease (58). It has also been associated with increased level of oxidative stress. In order to find out whether oxidative stress is a moderator, it is important to conduct a subgroup analysis of all these conditions associated with it.

Question 5: Does plasma ω-3 fatty acid interact with ApoE ε4 allele in increasing the risk of cognitive decline?

H_{5i}: Plasma ω-3 fatty acids are protective against cognitive decline only among subjects with at least one ApoE ε4 allele.

Question 6: Is there a similar interaction with other oxidative-stress inducing conditions?

H_{6i}: Plasma ω-3 fatty acids are protective against cognitive decline only at high levels of oxidative stress.

C. Rationale

Cognitive impairment is a major health concern that affects loss of independence in basic daily activities in older age and thus efforts should be devoted to preventing its occurrence. Hypertension, through its well-recognized contribution to the development of macroscopic cerebrovascular lesions, might equally well be expected to predispose to the development of more subtle cerebral processes, based on arteriolar narrowing, allied microvascular pathological features, or both, in due time leading to cognitive impairment, and finally overt dementia. This hypothesis has been confirmed by a wealth of epidemiological studies over the past two decades. However, the effect of ω-3 fatty acids on cognitive functioning has only been assessed at the biological and biochemical levels so far, and there is a paucity of epidemiological evidence in this regard.

The combined synergistic effect of hypertension and low dietary ω-3 fatty acid intake in increasing the risk of cognitive decline is also a biologically plausible phenomenon. ω-3 fatty acids have been historically associated with reduced risk of cardiovascular disease including stroke and coronary heart disease. They were also linked with improved insulin sensitivity, reduced risk of dyslipidemia, a hypocoagulable profile, improved pulmonary function and reduced risk of major depression among other health benefits. Because many of these conditions, including hypertension, were related to increased oxidative stress which in turn causes neuronal loss and cognitive impairment among older adults, it is essential to unveil any putative interaction between these conditions and the ability of this class of fatty acids to fulfill their beneficial effects. An additional genetic factor -- having an ApoE ε4 allele -- has been consistently associated with increased risk of cognitive decline and is now one of the main biomarkers for fast progression into Alzheimer's disease. It has also been associated with increased level of oxidative stress (402). In order to find out whether oxidative stress is a moderator, it is important to conduct a subgroup analysis of all these conditions associated with it.

Chapter 4

METHODS

A. Overview of Methods

The Atherosclerosis Risk in Communities (ARIC) is a prospective cohort study conducted between 1987 and 1998, which aimed at investigating the etiology of atherosclerosis and its clinical sequelae and variation in cardiovascular risk factors, medical care, and disease by race, sex, place, and time. In each of four US communities--Forsyth County (NC), Jackson (MS), suburbs of Minneapolis (MN), and Washington County (MD)-- 4,000 adults aged 45-64 years were examined four times so far, three years apart (total number at visit 1 was around 16,000). ARIC had coordinating, ultrasound, pulmonary and electrocardiographic centers and three central laboratories. Three out of the four cohorts represented the ethnic mix of their communities, while at Jackson (MS) only African-American residents were recruited. Examinations included ultrasound scanning of carotid and popliteal arteries; lipids, lipoproteins, and apolipoproteins assayed in the Lipid Laboratory; and coagulation, inhibition, and platelet and fibrinolytic activity assayed in the Hemostasis Laboratory. Surveillance for coronary heart disease involved review of hospitalizations and deaths among community residents aged 35-74 years. ARIC aimed to study atherosclerosis by direct observation of the disease and by use of modern biochemistry (403). To ensure that correct contact information was maintained, an annual follow-up was done.

This follow-up interview had also other functions such as ascertaining vital status and other interim medical events (mainly hospitalization, and new cardiovascular symptoms). Efforts were also made to identify cohort deaths before annual interviewing through frequent review of death certificates. When deaths were ascertained, a mortality interview was conducted at an appropriate time.

Participants who completed at least part of the baseline examination were followed and, if alive, invited to subsequent ARIC examinations. This excluded enumerated residents who completed the home interview, but did not sign the informed consent form at the field center. Participants did not have to still live in the community to participate in subsequent annual follow-up interviews or examinations. The scheduling of visits 2 through 4 were made in conjunction with the annual contact in the fourth contact year. The optimal time frame for scheduling was within 30 days of the participant's annual contact target date. As anticipated, most of the field center visits were completed within at least 90 days. However, if the participant for instance could not complete visit 2 within this window, it was still possible for visit 2 to be completed at any time during contact years 4 through 6. However, the contact year for visit 2 was labeled "year 4" regardless of when examination was conducted. The same principles applied to visits 3 and 4.

The ARIC visit 2 examination consisted of the following general components and they were administered by trained interviewers:

- *Informed consent*
- *Reception*: Greeted participant, determined fasting status, verified identifying information, obtained tracing data; collected medications.
- *Sitting blood pressure*: Obtained sitting blood pressure before the participant had blood drawn.
- *Anthropometry*: Measured weight, frame size, skin folds.
- *Venipuncture*: Obtained blood samples for all laboratory tests. Laboratory tests that had been conducted routinely were: Total cholesterol, LDL cholesterol, HDL cholesterol, Triglycerides, Hematocrit, Hemoglobin, White blood cell count, platelet count, magnesium, sodium, potassium, creatinine, uric acid and glucose.
- *Snack*

- *ECG*: Obtained a 12 lead ECG
- *Interview*: Collected sociodemographic, cognitive function, psychosocial, and selected medical, personal and family history data.
- *Physical exam*: Obtained a brief systems review on each participant including neck, neurological (stroke, TIA exam), chest and lungs, heart, and extremities. Verified reported history of possible CVD.
- *Pulmonary function*: Obtained spirometric measurements of timed pulmonary function (FVC, FEV_1) and inspiratory pressure (MIP).
- *Ultrasound*: Obtained B-mode scan and arterial wall distensibility measurements in carotid arteries. Measured heart rate and blood pressure changes as participant arised from supine position.
- *Medical Data Review*: Ascertained the completeness of the exam and verified abnormal results. Reviewed results of the medical history and exam with the participant. Referred participant for diagnosis or treatment elsewhere if appropriate.
- *Exit interview*: Returned medication; thanked participant.

Similar data were obtained in subsequent visits, with minor differences. Since we are mainly interested in the cognitive outcome at visits 2 and 4, we will discuss in further detail how this concept was operationalized in the data and how we will use it to construct our measure of cognitive decline. Data were archived using SAS electronic databases, which were later converted to be used by other software programs. Access to these databases was possible after a manuscript proposal was submitted and approved by the ARIC committee. Each database was then requested as needed from the coordinating center (http://www.cscc.unc.edu/aric/). Special requests for databases that were not part of the entire ARIC investigation included data on plasma fatty acids and genetic data on ApoE genotypes.

B. Design

B.1. Subject Identification & Sampling

The ARIC cohort who was first recruited between 1987-89 included 15,792 individuals between the ages of 45 and 64 years, of which 8,985 were women. The study participants were recruited using probability area sampling.

Ethnic diversity varied tremendously between Jackson county where the population chosen was exclusively African American, Forsyth County where 14% were African American and the other two communities where the population was predominantly white. The present study focused on examination visits 1, 2 and 4 which were carried out in the periods 1987-89, 1990-92 and 1996-98 respectively, although visit 3 data was sought in a few cases. In fact, while all exposures and most covariates were measured at visits 1 and 2, the outcome (i.e. cognitive decline) was measured between visits 2 and 4 and the main effect modifier (i.e. hypertension) between visits 1 and 4. Although this approach lead to censoring of the original sample which was selected at baseline, it was adopted by other investigators who used the ARIC dataset for a similar purpose. Previous analysis carried out by Alves de Moraes and colleagues (44) shows that 11,320 individuals had complete follow-up between visits 2 and 4. In addition, 234 had missing cognitive test scores at either visit. Therefore, the final study population at risk of cognitive decline should consist of 11,086 individuals. For the present study, we used the same study population by Alves de Moraes and colleagues (44) without excluding TIA or stroke cases (n=567) or subjects on psychotropic drugs (n=2,461). However, our study population was restricted further to older adults aged around 55 years or more at visit 2. To have a round number at visit 1, we restricted the population to those aged 50+ at baseline. Hence, it is expected that about half of the former population was eligible for our present study, since the mean age at visit 2 of the 8,058 individuals selected by Alves de Moraes and colleagues (44) was 56.7 with a SD of 5.6. There are two main reasons for restricting this population: **(1)** Cognitive decline, particularly dementia, is highly unlikely to occur before the age of 60 years, as shown by a wealth of previous literature (404) ; **(2)** The effect of hypertension on cognitive decline as shown by Alves de Moraes and colleagues (44) was much more pronounced among the population that was aged over the mean (i.e. older than 53 years at visit 1).

The study population selected as previously described was used for answering question 1. For question 2, a subset of these subjects, namely whites selected to participate in the Minneapolis field center, was abstracted since these are the subjects with complete data on plasma fatty acids. It is estimated that their study size would range between 2,000 and 2,500 depending on how balanced the allocation remains between the four communities when the exclusion and inclusion criteria are applied for the first study question. **Table 4.1** shows the preliminary analyses and the estimated number of subjects available for analysis to answer each of the questions. The numbers in "bold" are of primary interest, namely: **2,834** is the number of subjects who are 50 years of age or older with complete data on plasma and dietary fatty acids (these subjects were used for a validation sub-study); **7,817** is the total number of eligible subject with complete data on outcome and dietary exposure (they were used to answer the first question); **2,253** is the total number of eligible and available subjects to answer question 2 relating plasma fatty acids to cognitive decline.

B.2. Outcome Assessment

B.2.1. Screening Instruments

Three measures of cognitive functioning were made for visits 2 and 4 of the ARIC study, and these measures relied on the following instruments: Delayed Word Recall Test (DWRT) (167); the Digit Symbol Substitution portion of the Revised Weschler Adult Intelligence Scale (DSST/WAIS-R) (405), and Word Fluency Test (WFT) of the Multilingual Aphasia Examination, also know as the controlled oral word association (170). They are the same instruments that were previously used by Knopman and Alves de Moraes and colleagues (44, 150), as well as others (197, 406).

(1) *Delayed Word Recall Test (DWRT)*: This screening tool assesses verbal learning and recent memory. It requires from the respondent to recall 10 common words after a 5-minute interval during which another test is administered.

The ten words used in ARIC were: chimney, salt, harp, button, meadow, train, flower, finger, rug and book. To standardize the elaborate processing of words to be recalled, individuals are asked to compose sentences with the words that are presented. Test scores may range between 0 and 10 words recalled and the time limit for recall is set at 60 seconds. The 6-months test-retest reliability of DWRT was previously shown to be high among 26 normal elderly individuals (Pearson correlation coefficient, r=0.75) (167).

(2) *Digit Symbol Substitution (DSST/WAIS-R):* This test is a paper-and-pencil test requiring timed translation of numbers 1 through 9 to symbols using a key. The test measures psychomotor performance and is relatively unaffected by intellectual ability, memory, or learning for most adults(170). It appears to be a sensitive and reliable marker of brain damage(407). The test score can range between 0 and 93 and it refelects the correctly translated number of digit-symbol pairs within a time limit of 90 seconds. Short-term test-retest reliability over 2-5 weeks has been found to be high in individuals aged 45-54 years (r=0.82); (405).

(3) *Word Fluency Test (WFT):* This test requires subjects to record as many words as possible using the letters F, A and S and to list these words, the subject is given only 60 seconds per letter. The total score corresponds to the total number of words generated during these three trials. The test is particularly sensitive to linguistic impairment (170, 408) and early mental decline in older persons (409). It is also a sensitive marker of damage in the left lateral frontal lobe (170, 408). The immediate test-retest correlation coefficient based on an alternate test form has been found to be high (r=0.82); (410).

The DWRT, WFT, and DSST/WAIS-R were administered by trained interviewers and since all interviews were tape-recorded, quality checks were feasible for at least a random sample to ensure acceptable performance.

Table 4.1 Eligible population as enumerated according to specific criteria; ARIC cohort

	All centers	**Minneapolis**	
All those with complete data on age at visit 1	15,792	4,008	
All those aged 50+ at visit 1	11,557	2,928	
Age			
50-54	35.5		
55-59	33.3		
60+	31.2		
% Female	53.8		
% White	74.4		
All those aged 50+ at visit 1 (white population)	8,598	2,902	
All those aged 50+ at visit with data on plasma fatty acids	n/a	2,878 (white population)	
All those aged 50+ at visit 1 who had complete data on plasma and dietary fatty acids	n/a	**2,834** (white population)	
Age			
50-54		**37.7**	
55-59		**33.3**	
60+		**29.0**	
% Female		**49.5**	
% White		**100%**	
All those aged 50+ at visit 1 who had complete data on dietary fatty acids	11,307	2,834	
All those aged 50+ at visit 1 who had complete data on <u>dietary</u> fatty acids and cognitive functioning variables	**7,817**	All those aged 50+ at visit 1 (in the Minneapolis center) who had complete data on <u>plasma</u> fatty acids and cognitive functioning variables.	**2,253** (white population)
Age			
50-54	**37.2**		**39.3**
55-59	**33.5**		**33.0**
60+	**29.3**		**27.2**
% Female	**54.6**		**50.7**
% White	**81.5**		**100%**

B.2.2. Analytic Plan

For the purpose of assessing cognitive decline, there are several options at hand. However, the one that we chose is an innovative way that attempts to create a single measure of decline and would combine all three screening tool scores together. In addition, we assessed decline in separate domains of cognition. Cutoff points were determined for decline in each domain of cognition using the Reliable Change Index (RCI) method to correct for measurement error and practice effects (411). RCI is defined as $((X_2-X_1)-(M_2-M_1))/S.E.D.$, where X_1 is the individual's score at baseline, X_2 the individual's score at follow-up, M_1 and M_2 are the group mean pretest and follow-up scores respectively, and S.E.D. the observed standard error of the difference scores. Scoring below an RCI of -1.645 was regarded as a "statistically reliable" deterioration in the test scores. A composite measure of the three RCIs to assess global cognitive decline (GCD) was created using principal components analysis (PCA). In multivariate analysis, control was done on baseline cognitive score in its continuous form (assessed at visit 2) on that particular instrument. For models with GCD as the main outcome, control was done on a measure of global baseline cognitive functioning (GBCF) which reduces the three baseline scores into a single component using PCA. All analyses was done using STATA ver. 8.2 (412).

B.2.3.Principal Components Analysis

To better understand Principal Component analysis (PCA), I have derived information from three main references (413-415). However, other sources have been used as well as needed. Principal components analysis has been historically confused with factor analysis. Assuming that the elements of the vector x of manifest variables are standard scores, we can distinguish principal components analysis from factor analysis with respect to several aspects:

Table 4.2 Comparison table of PCA and FA

Principal Components Analysis (PCA)	Factor Analysis (FA)
Better fit to raw data Better fit to Manifest Variable (MV) variances	Better fit to MV intercorrelations
No Latent Variables (LVs).; Component scores are determinate, but components are not LVs.	Factor scores are indeterminate, but factors are interpretable as LVs.
Not a testable model.	Testable model.
Residuals are correlated.	Unique factors uncorrelated.
Useful for data reduction.	Useful for understanding structure of MV intercorrelations.

Source: McCallum, 2004.

Despite these clear differences, both methods are based on very similar fundamental models which can be written as follows:

FA model: $x_{ij} = \mu_j + \lambda_{j1}z_{i1} + \lambda_{j2}z_{i2} + \ldots\ldots\ldots\ldots\ldots \lambda_{jm}z_{im} + u_{ij}$
PCA equation: $x_{ij} = \mu_j + \lambda_{j1}z_{i1} + \lambda_{j2}z_{i2} + \ldots\ldots\ldots\ldots\ldots \lambda_{jm}z_{im} + e_{ij}$

Hence, for both models, a score on a manifest variable j obtained by an individual i can be written as a function of the mean score on manifest variable j, a set of unmeasured variables z_{i1} called factor scores with their corresponding factor loadings, and a residual or unique factor portion uij (for FA) and eij (for PCA).

The main difference is that factor scores are indeterminate in the case of FA, while for PCA an estimate of factor scores can be obtained and these factors are therefore not latent variables. The unique factor portions in FA are uncorrelated while in PCA they may be correlated.

The PCA equation can be expressed as follows:

$x = \ddot{\Lambda}\,\ddot{z} + \ddot{u}$; where $\ddot{\Lambda}$ is $(p \times m)$ matrix of principal components loadings, x is a $(p \times 1)$ matrix of manifest variables, $\ddot{z}$ is a $(m \times 1)$ vector of principal components scores and $\ddot{u}$ is a $(p \times 1)$ vector of unique components.

In PCA, unlike FA, $\ddot{z}$ can be expressed as a linear combination of manifest variables x_j, for each individual i, as follows:

$\ddot{z}$ = B'x; where B': m x p.

The component scores, $\ddot{z}$, are defined so as to maximize the average of squared multiple correlations of manifest variables on *m* components, as follows:

$$\text{ASMC} = \frac{1}{p}\sum_{j=1}^{p} r^2(x_j.\ddot{z}_1, \ddot{z}_2, ..., \ddot{z}_m)$$

Where $r^2(x_j.\ddot{z}_1, \ddot{z}_2, ..., \ddot{z}_m)$ is the squared multiple correlation of each manifest variable on the *m* components and *p* is the number of manifest variables. Hence, the *m* component scores are chosen so as to predict the p manifest variable scores as precisely as possible. The aim of principal components is data reduction with minimal loss of information concerning the original variables.

The solution for B', as it turns out is:

$B' = (\ddot{\Lambda}' \ddot{\Lambda})^{-1} \ddot{\Lambda}'$

$\text{Cov}(\ddot{z}, \ddot{u}') = 0$

Hence, $z = [(\ddot{\Lambda}' \ddot{\Lambda})^{-1} \ddot{\Lambda}']x$

The main distinctive difference between FA and PCA is as follows:

In FA: $R - \Lambda\Lambda'$: off-diagonals represent correlation residuals and these are minimized, while diagonals represent unique variances.

In PCA: $R - \ddot{\Lambda}\ddot{\Lambda}'$: off-diagonals represent correlation residuals, but the diagonals are the ones that are minimized and they represent residual variances.

In general, factor loadings are overestimated in PCA as compared to FA and hence PCA should not be used for factor analytic purposes but rather for data reduction purpose. The underlying principle of components analysis is different from that of factor analysis.

Therefore, one cannot be used as a substitute for the other. But, in a larger context of practical research, both have legitimate uses. There are situations in which the component scores may be preferred to the factor scales. In particular, if the objective is some simple summary of information contained in raw data without recourse to factor analytic assumptions, the use of components scores has a definite advantage over factor scaling. The principal components are no more than exact mathematical transformations of the raw variables. Therefore, it is possible to represent the components exactly from the combination of raw variables, and we can speak of component *scores*, instead of *scales* or *estimates*. The scores are obtained by combining the raw variables with weights that are proportional to their component loadings as follows:

$$\text{Component Score} = \sum_{i}[(\lambda_{ij} / \gamma_{j})X_{i}]$$

Where λ_{ij} are the component loadings, γ_{j} are the eigenvalues associated with each component, and Xi are the manifest variable scores. Division by the eigenvalue which is a function of the variance explained by each component ensures that the resulting component score had a mean of zero and a standard deviation of 1 (414).

It is important to note, that even though factors in factor analysis are indeterminate latent variables, there are a number of methods that were developed to estimate *factor scores*. However, factor scores are distinct from component scores and their estimation does not lead to maximization of the percentage variance explained but rather of the minimization of correlation residuals. Hence, it is more efficient to use component scores rather than factor scores as a data reduction procedure.

C. Classification of Exposure

C.1. Exposures of Interest

C.1.1. Dietary ω-3 fatty acids

Usual dietary intake was estimated from an interviewer-administered semi-quantitative food frequency questionnaire (FFQ) modified from a 61-item questionnaire developed and validated by Willet and colleagues against multiple food records among a sub-sample of the Nurse's Health Study cohort. Results of the validation study suggested that for all nutrients considered there was only up to 3% extreme quintile misclassification and that overlap between the upper two quintiles and lower two quintiles between the two methods was >70%. In addition, energy adjustment improved validity especially for nutrients that contribute most to caloric intake, mainly fats (416). On visits 1 and 3 of the ARIC study examinations, and using the Willet 61-item FFQ, the subjects were asked how often, on average, they had consumed certain foods in portions of a specified size (e.g., 85 to 113 g [3 to 4 oz] of canned tuna fish) during the preceding year. There were nine possible responses, ranging from "almost never" to "more than six times per day." Daily intake of nutrients has been calculated by multiplying the nutrient content of each food in the portion specified by the frequency of daily consumption and summing the results. The nutrient content of each food was obtained from the Harvard nutrient data base for which the primary source was the Department of Agriculture handbook (417). Fish consumption, the main dietary source of long-chain ω -3 fatty acids was estimated by summing the reported consumption of three items: 85 to 113 g of canned tuna fish, 85 to 142 g (3 to 5 oz) of dark-meat fish (e.g., salmon, mackerel, swordfish, sardines, or bluefish), and 85 to 142 g of other fish (e.g., cod, perch, or catfish). The eicosapentaenoic acid and docosahexaenoic acid content of these foods was estimated to be 190 and 500 mg, respectively, for tuna fish, 560 and 780 mg for dark-meat fish, and 240 and 460 mg for other fish (357).

In our present study, dietary fatty acids were analyzed from the food frequency questionnaire from visit 1, after assessing the magnitude of measurement error and controlling for its effect as will be discussed in the "Data analysis" sub-heading. Although DHA+EPA+ω-3DPA expressed as % of total energy intake is the main exposure of interest, other variants of exposure were assessed as well and these are presented in **Table 4.3**.

When relationships with disease are analyzed, nutritional factors may be examined in terms of absolute amount (crude intake) or in relation to total caloric intake. The analytic approach depends on both the nature of the biologic relationship and the public health considerations. If a nutrient is metabolized in approximate proportion to total caloric intake (such as the macronutrients and some vitamins), nutrient intake is most likely biologically important in relation to caloric intake. In fact, intake of most nutrients in free-living populations tend to be positively correlated with total energy intake (418-420).

Correlations were shown to be particularly strong for fat, protein and carbohydrates (which contribute to energy intake). All other nutrients were also shown to be moderately correlated with energy intake even though they did not contribute to energy. For instance, correlations were 0.24 for fiber, 0.25 for vitamin A, and 0.19 for vitamin C. This is complicated by the observation that dietary composition is also interrelated with total caloric intake whereby a person whose caloric intake is low on average tends to have a higher intake of fiber than a person whose caloric intake is high. These observations create the need to control for caloric intake when looking at associations between specific nutrients and disease in epidemiologic studies.

Table 4.3 Operationalization of dietary fatty acid intake in the causal models[28]

	Exposure 1[29]	*Exposure 2*	*Exposure 3*	*Exposure 4*
Model A	**{20:5+22:5+22:6ω-3}**	20:3+**{20:4}**+22:4+22:5 ω-6	**{18:3+18:4ω-3}**	**{18:2}**+18:3ω-6
Model B	**{18:3+18:4ω-3}**	**{20:5+22:5+22:6ω-3}**	**{18:2}**+18:3ω-6	20:3+**{20:4}** +22:4+22:5 ω-6
Model C	**{18:3+18:4+20:5+22:5 +22:6ω-3}**	**{18:2}**+18:3+ 20:3+**{20:4}**+22:4+22:5 ω-6	…	…
Model D	[exposure 1/ exposure 2] in model 1	**{18:3+18:4ω-3}**	**{18:2}**+18:3ω-6	…
Model E	[exposure 1/ exposure 2] in model 2	…	…	…
Model F	[exposure 3/ exposure 4] in model 1	**{20:5+22:5+22:6ω-3}**	20:3+**{20:4}**+22:4 +22:5 ω-6	…
Model G	**{20:5+22:5+22:6ω-3}** in mg/day	20:3+**{20:4}**+22:4+22:5 ω-6 in mg/day	**{18:3+18:4ω-3}** in mg/day	**{18:2}**+18:3ω-6 in mg/day

Because a person's long-term total caloric intake is largely determined by body size, physical activity, and metabolic efficiency, even relatively small changes in caloric intake cannot be made unless changes in weight or physical activity occur. In the absence of such changes, therefore, most alterations in absolute nutrient intake must be accomplished by changing the composition of the diet rather than the total amount of food. For this reason among others, Hegsted (358) made recommendations for fat intake not to exceed 30% of total caloric intake. Therefore, from a

[28] In this table, fatty acids between braces and bolded are available in the FFQ data whereas the others were not analyzed. However, the sum of the missing fatty acids as % of total energy <0.005% according the literature and this value was imputed as 0.003% for ω-6 18-C UFAs and 0.002% for ω-6 20 and 22-C HUFAs.

[29] Note that exposure 1 is the main exposure and is the one which will be interacted with "hypertension". Control is made on other fatty acids when appropriate.

practical or public health standpoint, nutrient intake in relation to total caloric intake (i.e. compositional aspects of the diet) is most relevant.

For this reason, in epidemiologic studies, nutrient intakes adjusted for total energy intake, rather than absolute nutrient intakes, are of primary interest in relation to disease risk. This process of adjusting for energy intake can be viewed in analogy to experimental animal studies where the effect of a single nutrient on disease outcome can only be ascertained under *isocaloric* conditions.

When total caloric intake is associated with disease, the interpretation of individual nutrient intake is complex, and the consequence of failing to account for energy intake may be far more serious. In fact, in nearly every study looking at diet and coronary heart disease, subjects who subsequently develop disease have lower caloric intake on average when compared to those who remain free of disease. As a result, intake of specific nutrients also tends to be lower among cases than among non-cases. This inverse association between caloric intake and coronary heart disease can be biologically related to the fact that those who develop the outcome were physically less active and hence had lower caloric intake. Hence, in order to look at the effect of a specific nutrient on an outcome that is related to caloric intake, one should energy adjust this relationship (363). There are several means by which one can adjust by energy the relationship between a specific nutrient and a disease outcome. These can be summarized as follows:

Table 4.4 Alternative disease risk models for addressing the correlation of specific nutrient intakes with total energy intake in epidemiologic studies

Disease Risk Model	(Method) Definition
Model A	(Residual Method) Disease=b_1 Nutrient residual [a]
Model B	(Residual Method) Disease=b_1 Nutrient residual + b_2 Nutrient residual
Model C	(Standard Multivariate Method)
	Disease = b_3 Calories + b_4 Nutrient
Model D	(Energy Partition Method)
	Disease = b_5 $Cal_{Nutrient}$ + b_6 Cal_{other}
Model E	(Multivariate Nutrient Density Model)
	Disease = b_7 Nutrient/Calories + b_8Calories

[a] "Nutrient residual" is the residual from the regression of a specific nutrient on calories.
[b] $Cal_{nutrient}$ represents calories provided by the specific nutrient.
[c] Cal_{other} represents calories from sources other than the specific nutrient.
Source: (363)

Because there was high correlation between total energy intake and individual nutrient intakes estimated by FFQ, nutrients must be energy-adjusted. There are several methods used for this purpose, the simplest one being the nutrient density method in which each nutrient is expressed as % energy intake by dividing it by the total energy. In our case, however, although caloric intake is added to the model, the main exposure itself varied according to the particular model. A main transformation done to the Nutrient/Calories term is its multiplication by the caloric density of fat (9 Cal/g) and by the factor of 100 to obtain the % of total calories from a specific type of fatty acids. For ratio of fatty acids variables (e.g. total ω-3/total ω-6), these are interpretable as ratio of absolute intake of ω-3 over absolute intake of ω-6 fatty acid, controlling for other nutrients and for total energy intake among other covariates. Hence, the model that we ran closely resembles Model E although the main variable has undergone transformation, mainly multiplication by a factor of 900.

According to Willet (416), the more sophisticated residual method yields similar results for total fat as compared to the nutrient density method of energy adjustment (421). After verifying linearity in the logits, each of these continuous exposure variables were entered as standardized z-scores into multivariate models. This is done by subtracting each value from its mean and dividing the difference by the corresponding standard deviation.

C.1.2. Plasma ω-3 fatty acids

Twelve hour fasting blood was collected according to the ARIC study wide protocol. The Minneapolis field center conducted the analysis for visit 1 blood specimens among the white segment of the study population in that center.

Even though the procedure is described in great detail by Shahar and colleagues (422), I attempted to summarize as follows: First of all, the sample for fatty acid analysis was collected in a 10-ml tube containing ethylenediamine tetraacetic acid (EDTA). The tube was refrigerated and sent to the study clinic by courier. Upon arrival, the blood was centrifuged at 800 × g for 10 minutes. The plasma was separated and divided into two 1.5 ml aliquots, and frozen at -70 ^{o} C. In order to use the frozen plasma for fatty acid analysis, 0.5 ml of plasma was needed and extracted using 0.5 ml of methanol followed by 1.0 ml of chloroform under a nitrogen atmosphere. The lipid extract was then filtered to remove protein. The phospholipids and cholesterol ester fractions were separated by using thin-layer chromatography, silica gel plate and two-stage mobile-phase development, with use of 80:20:1 (vol/vol) and 40:60:1 (vol/vol) petroleum ether, diethyl ether, and glacial acetic acid, respectively. The chromatography plate was then dried between exposure and development solvents, and the second mobile phase was allowed to migrate only half the length of the plate. After the plate was redried, one lane was sprayed with dichlorofluorescein to visualize the phospholipids, cholesteryl ester, triglyceride, and fatty acid bands under ultraviolet light. The phospholipids and cholesteryl ester bands were scraped into separate test tubes and the lipids were converted to methyl esters of fatty acids by boron trifluoride catalysis. The methyl esters were then separated and measured with a Model 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a 30-m FFAP WCOT glass capillary column (J & W Scientific, Folsom CA) and a flame ionization detector. The identity of 28 individual fatty acid peaks was revealed by gas chromatography and determined by comparing each peak's retention time to the retention times of fatty acids in synthetic standards of known fatty acid compositions. The relative amount of each fatty acid (as a percent of all fatty acids) could be calculated by integrating the area under the peak and dividing the result by the total area for all fatty acids (×100).

To minimize transcription errors, the data from gas chromatogram was transferred electronically to a computer (Digital equipment Corporation, Minneapolis, MN) for data analysis. Out of the 28 fatty acids that were analyzed both in the phospholipid and cholesteryl ester fractions, the ones that are of highest interest in the present study are DHA and EPA (two long-chain 20-and 22-C ω-3 fatty acids) in contrast to AA and other long chain 20-C ω-6 fatty acid maintained in plasma phospholipids and cholesteryl esters. In addition, the ratio of (DHA+EPA)/(long chain ω-6 fatty acids) fatty acids is also of high priority. The estimated short-term reliability coefficients for DHA and EPA were, according to a study by Ma and colleagues (423), around 0.5 and 0.3. The values of the biomarkers and ratios of biomarkers were subtracted from their means and divided by their respective standard deviations to obtain a z-score. As was discussed for the dietary exposure, the plasma values were entered into multivariate models in their continuous forms if the linearity of the logits assumption can be verified through non-parametric exploratory data analyses. Otherwise, categorization were done using cutoff points chosen according to the findings of that analysis. In addition to testing the second hypothesis, plasma fatty acids were used to validate dietary fatty acid intake by conducting a validation sub-study and applying its result to the overall eligible population, as will be discussed later on under "Data analysis". It is worth noting that the main difference between the plasma markers of interest and the dietary fatty acids that are assessed is that the latter include Linolenic acid as an important source of long-chain fatty acids in the brain. However, since the level of Linolenic acid in plasma does not necessarily affect the level of DHA and EPA in the brain, it was not be assessed as an exposure of interest in plasma, as previously asserted by Yamamoto and colleagues (21). Table 4.5 shows operationalization of the main exposure as well as the other fatty acids that were controlled for in the analysis for each causal model.

Table 4.5 Operationalization of plasma fatty acid in the causal models[30]

	Exposure 1[31]	*Exposure 2*	*Exposure 3*	*Exposure 4*
Cholesteryl esters				
Model A	**{20:5+22:6ω-3}**	**{20:3+20:4+22:4+22:5 ω-6}**	**{18:3}+**18:4ω-3	**{18:2+18:3ω-6}**
Model B	(Exposure 1 /Exposure 2) in model 1	**{18:3}+**18:4ω-3	**{18:2+18:3ω-6}**	…
Phospholipids				
Model C	**{20:5+22:6ω-3}**	**{20:3+20:4+22:4+22:5 ω-6}**	**{18:3}+**18:4ω-3	**{18:2+18:3ω-6}**
Model D	(Exposure 1 /Exposure 2) in model 1	**{18:3}+**18:4ω-3	**{18:2+18:3ω-6}**	…

C.2. Covariates

Most covariates to be considered as potential confounders were measured at visits 1 or 2, although some were defined according to criteria that spanned all four visits. Covariates can be subdivided into socio-demographic, genetic, health behaviors, nutritional, and co-morbid conditions or medications. Age (measured at visit 1), gender, ethnicity and education were all reported by respondent. One main genetic factor, Apo E genotype, was considered in the analysis and categorized as 0: "does not have an ε4 allele"; 1:"has at least one ε4 allele". Among the behavioral factors (all measured at visit 1), smoking was represented on a three-level categorical scale, namely: 0"never smoked", 1"smoked previously" and 2"current smoker". FFQ derived values of alcohol (grams/day) and caffeine (mg/day) were considered as well. Finally, physical activity was assessed by interview using a questionnaire developed by Baecke and colleagues, including 16 items about usual exertion (424). An index of physical activity was derived at visit 1, summing sports, work and leisure indices which ranged from a score of 1 (low) to 5 (high). The total score was shown to be a valid and reliable measure of physical activity (425).

[30] In this table, fatty acids between braces and bolded are available in the plasma fatty acids data whereas the others were not analyzed. However, the percentage of these fatty acids in both cholesteryl esters and phospholipids are considered as negligible compared to the other fatty acids that were analyzed.

[31] Note that exposure 1 is the main exposure and is the one which will be interacted with "hypertension".

In terms of nutritional factors, body mass index at visit 1 was computed by dividing weight in kilograms by the height-squared (in square meters). Baseline intake of antioxidants and other micronutrients (mainly Vitamins B_{12} and E) was considered as well. A number of co-morbid conditions were deemed as effect modifiers, the most important of which is hypertension. For our purpose, an algorithm was used to define hypertensive status, using two of the three criteria that were used by Knopman and Alves de Moraes and colleagues (44, 150). Our main effect modifier, hypertension, was operationalized using measured systolic and diastolic blood pressure at each visit as well as use of anti-hypertensive medication over the past two weeks. Blood pressure levels were calculated as the average of the second and third of three consecutive measurements with a random-zero sphygmomanometer. The cutoff point often used for hypertension is ≥140 mm Hg. for SBP and ≥90 mm Hg. for DBP. Hypertensive individuals were defined as those who screened positive for measured hypertension at either visits 1 through 4 or were taking anti-hypertensive medication over the past two weeks prior to examination on any of those visits.

Other co-morbid conditions considered as putative effect modifiers included: Stroke or TIAs, type II diabetes mellitus (defined as fasting blood glucose ≥140 mg./dl or self-reported diabetes or use of glucose lowering medication) and dyslipidemia (fasting blood HDL-C<40 (men) or <50 (women) and TAG>150 mg/dl as recommended by NCEP (426)) at any visit, hypercoagulable profile (upper quintile of at least two of: fibrinogen, vWF and factor VIII), poor pulmonary function (ratio $FEV_1/FVC \leq 0.70$ as measured by a spirometer at visits 1 or 2), and depressive symptoms at visit 2 using a 21-item vital exhaustion scale.

The scale which was developed in the Netherlands, was found to be correlated with the Beck Depression Inventory (r=0.62) and is scored on a scale of 0-42, with positive responses scored as two; don't know scored as one and negative responses as zero, except for two questions where scoring is reversed (427, 428). Use of medications was measured for descriptive purposes. Medications considered included statins or medications known to lower cholesterol, NSAIDs and psychotropic drugs, screened at all visits. The association of these covariates with our outcome was documented by several secondary analyses of the ARIC cohort (150, 197, 216, 406). **Table 4.6** shows the detailed operationalization of each covariate as well as the onset and period of exposure considered.

Table 4.6 Operationalization of covariates and effect modifers to be potentially included in the analysis

Covariate	Onset of follow-up	Period of follow-up	Definition	Categories
SOCI-D				
AGE	Visit 1	n/a	Age of eligible subject at visit 1 (in years)	As it is; 50-65
GENDER	n/a	n/a	Gender of eligible subject	1. Male; 2. Female
ETHNICITY	n/a	n/a	Ethnicity of eligible subject	1. White; 2. Non-white
EDUCATION	Visit 1	n/a	Educational attainment of eligible subject	1. Less than HS 2. HS+
GENETIC				
APOEε4 GENOTYPE	n/a	n/a	ApoE ε4 genotype	0. No ε4 allele 1. At least one ε4 allele
BEHAV				
SMOKING STATUS	Visit 1	Visit 1	Smoking status of eligible subject	0. Never smoked 1. Smoked previously 2. Current smoker
ALCOHOL CONSUMPTION	Visit 1	Visit 1	FFQ-derived value of ethanol (g./day)	As it is
CAFFEINE CONSUMPTION	Visit 1	Visit 1	FFQ-derived value of caffeine (mg./day)	As it is
PHYSICAL ACTIVITY	Visit 1	Visit 1	Physical activity assessed by three sub-scales: work, leisure and sports. Sum of the sub-scales is computed.	As it is (0-15)

Covariate	Onset of follow-up	Period of follow-up	Definition	Categories
NUTR				
BMI	Visit 1	Visit 1	Body mass index of eligible subject: directly measured as weight(kg.)/Height^2 (in m^2).	As it is
TOTAL CALORIC INTAKE	Visit 1	Visit 1	FFQ-derived value in KCal/day	As it is
VITAMIN A	Visit 1	Visit 1	FFQ-derived value in 1000 IUs/day	As it is
VITAMIN C	Visit 1	Visit 1	FFQ-derived value in mg./day	As it is
VITAMIN E	Visit 1	Visit 1	FFQ-derived value in mg./day	As it is
FOLATE	Visit 1	Visit 1	FFQ-derived value in mcg./day	As it is
VITAMIN B_6	Visit 1	Visit 1	FFQ-derived value in mg./day	As it is
VITAMIN B_{12}	Visit 1	Visit 1	FFQ-derived value in mcg./day	As it is
CO-MB				
HYPERTENSION	Visit 1	Visits 1-4	≥140 mm Hg. for SBP and ≥90 mm Hg. for DBP or taking anti-hypertensives.	0. no; 1. yes
STROKE or TIAs	Visit 1	Visits 1-4	History of Stroke or TIAs	0. no; 1. yes
DEPRESSION	Visit 2	Visit 2	21-item vital exhaustion scale	Continuous; sum of positive responses (Yes: 2; Don't know: 1; No: 0). Range: 0-42.
TYPE II DIABETES	Visit 1	Visits 1-4	Type II diabetes as assessed by self-report and/or blood glucose level >140 mg/dl and/or glucose lowering medication over past two weeks.	0. no; 1. yes

Covariate	Onset of follow-up	Period of follow-up	Definition	Categories
DYSLIPIDEMIA (NCEP)	Visit 1	Visits 1-4	Hypertriglyceridemia defined as TAG> 150 mg./dl, and/or HDL cholesterol < 40 mg./dl for men and <50 mg./dl for women on at least two visits.	0.no; 1. yes
POOR PULMONARY FUNCTION	Visit 1	Visits 1-2	ratio $FEV_1/FVC \leq 0.70$ as measured by a spirometer at visits 1 or 2	0. Normal; 1. Poor
HYPERCOAGULABLE PROFILE	Visit 1	Visit 1	Fibrinogen, vWF and Factor VIII values: Highest quintile on two out of three.	0.Low; 1. High
MEDIC				
USE OF PSYCHOTROPIC MEDICATIONS	Visit 1	Visits 1-4	Use of any of a list of psychotropic drugs (CNS, Anesthetic/analgesic or neuromuscular drugs)	0. no; 1. yes
USE OF ANIT-INFLAMMATORY DRUGS	Visit 1	Visits 1-4	Use of NSAIDs.	0. no; 1. yes
USE OF STATINS	Visit 1	Visits 1-4	Use of any of a list of drugs classified as statins (cholesterol lowering or known to lower cholesterol)	0. no; 1. yes
BASELINE COGNITIVE FUNCTIONING	Visit 2	Visit 2	Baseline cognitive functioning (global or domain-specific).	As it is

D. Quality Assurance/Quality Control

During the data collection phase, routine quality assurance was provided at each field center by means of observation by the local study coordinator. Protocol adherence and interviewing technique were reviewed biannually by Coordinating Center Field (CCF) center monitors. Deviations from protocols and possible remedial actions were discussed with study coordinators and staff.

Major deviations, if applicable, were brought to the attention of the Exam (EXM) Committee. In terms of training, study coordinators were centrally trained before visit 2 and were responsible for providing local staff training before visit 2 start up. The same procedure applied to visit 4. With participant approval, and as stated earlier, all interviews were taped for quality control. A non-systematic sample of interviews was reviewed by the supervisor. Technique and adherence to protocol are also monitored at least semi-annually by Coordinating Center Monitors; data quality is monitored by the quality control committee on a semi-annual basis.

In our present study, the following quality assurance efforts were be performed: (1) We looked for outliars in all exposure and outcome variables as well as covariates that are included in our models, (2) These outliars were deleted pairwise rather than listwise (3) Regression model assumptions were tested for adequacy using non-parametric methods and graphical representations. In particular the linearity of the logits assumption was tested for continuous exposure variables and normality of the exposure against the logits was also be verified.

E. Data Analysis

Statistical analysis was split into three parts: **(1)** Plasma fatty acids were used as a biomarker for dietary fatty acids in a validation sub-study spanning only the Minneapolis White population. **(2)** Using results from the validation sub-study, the association between dietary fatty acids and cognitive decline outcomes was corrected for measurement error for the entire study population (i.e. spanning all four centers); **(3)** Plasma fatty acids were considered as the main exposure of interest in relation to cognitive decline outcomes and analysis was done only on the White Minneapolis center study subjects. No measurement error correction was conducted for this part of the analysis.

E.1. Validation Sub-study

The complexity of dietary behavior has been contrasted with the simplicity of dietary assessment tools. Consequently, measuring usual intake of nutrients with semi-quantitative food frequency questionnaires (FFQ) in epidemiological studies as an exposure to common chronic diseases has been plagued by random and systematic errors (429). Since systematic errors generally cause bias in relative risk estimates derived from multivariate models, it is crucial to assess validity of intake measures, in order to enhance the interpretation of estimated diet-disease associations and to improve their translation into dietary recommendations (421, 430). One cost of measurement error in multivariate analyses is loss of statistical power. Assuming random error that is independent of true value of exposure and outcome *Y*, regression calibration is a technique which corrects for biases in causal models in situations where exposure variables are measured with error. The existence of a validation sub-study, where accurate and crude measurement methods are related by a second regression analysis, is assumed (431). Food frequency questionnaires (FFQ) have been historically validated against another dietary assessment method assumed to be more accurate such as multiple 24-hour recalls or food records of food intake (432-434). The problem attending this approach is that the same factors that affect these reference methods (*R*) may also affect the FFQ-based assessments (*Q*). This problem would make it impossible to presume independent random errors in the two methods, which in turn leads to over-estimation of the correlation between the reference method and the FFQ (435). Hence, to consider a method a gold standard, it should ideally be an accurate depiction of the truth and any error associated with it should be independent of errors that the test measure itself carries. Such restriction on measurement error associations makes biomarkers a desirable target for validation studies.

The present study aims at validating an FFQ for the estimation of essential fatty acid intake and biologically plausible combinations and ratios of these nutrients.

The study utilizes two reference measures: (i) an alloyed gold standard (*T**) based on a biomarker of specific fatty acid levels in plasma phospholipids and empirically derived biochemical equations that estimate true dietary intake (381-383). This alloyed gold standard takes the biochemical approach to validation (ii) Two instrumental biomarkers for the level of fatty acids in the cholesteryl ester (*M*) and phospholipids (*N*) fractions of plasma which were previously shown to be linearly related to dietary intake as assessed by more reliable reference methods such as multiple 24-hour recalls, food records or diet history (373-375, 436-440). These instrumental biomarkers take an epidemiological approach to validation. Findings from our study and its overall methodology may be used in subsequent analyses to adjust for measurement error in causal models linking intake of essential fatty acids and their biologically plausible ratios with disease outcomes.

While most epidemiological studies assume a linear relationship between dietary fatty acid intake and different biomarkers, the metabolic interactions of these fatty acids upon assimilation into different compartments suggest otherwise. Biochemical studies in the area of lipid research conducted by Lands and colleagues (381-383) suggest that the mixture of 20-C and 22-C highly unsaturated fatty acids (HUFAs) that are maintained in phospholipids of human plasma is related to the dietary intake of the four groups of fatty acids that were described earlier, expressed as percent of total energy intake. This relationship was empirically derived and is hyperbolic in nature among both humans and laboratory rats. The derived empirical equations were successful at reflecting the general metabolic selectivities that maintain fatty-acid composition in plasma phospholipids. The authors clearly stated that these equations can be used in reverse to estimate dietary intake of the ω-3 and ω-6 fatty acids by using the composition of the fatty acids that had been maintained in plasma phospholipids. The following competitive hyperbolic relationship reflecting saturable rate-limiting biochemical processes was used to derive these equations:

$$\text{Response} = \frac{V_{max}}{1+\frac{K_m}{en\%S}\left(1+\frac{en\%I}{K_i}\right)} \quad \textbf{(4.1)}$$

Where V_{max} in this case is 100, K_m and K_i are constants that are closely related to the type of dietary fatty acid (*S* or *I*) in question. In particular, the following three empirically derived equations (**4.2.1** through **4.2.3**) were used in our analyses:

$$pfh_6 = \left(1+\frac{C_6}{T_{6p}}\left(1+\frac{T_{3p}}{C_3}+\frac{T_o}{C_o}+\frac{T_{6p}}{K_S}\right)\right)^{-1} 100 \quad \textbf{(4.2.1)}$$

$$pfh_3 = \left(1+\frac{C_3}{T_{3p}}\left(1+\frac{T_{6p}}{C_6}+\frac{T_o}{C_o}+\frac{T_{3p}}{K_S}\right)\right)^{-1} 100 \quad \textbf{(4.2.2)}$$

$$pfh_6 = \left(1+\frac{PC_6}{T_{6p}}\left(1+\frac{T_{3p}}{PC_3}+\frac{T_{3p}}{HI_3}+\frac{T_o}{C_o}+\frac{T_{6p}}{K_S}\right)\right)^{-1} 100 + \left(1+\frac{HC_6}{T_{6H}}\left(1+\frac{T_{3H}}{HC_3}\right)\right)^{-1} 100 \quad \textbf{(4.2.3)}$$

Table A (Appendix A) shows definitions and values of each of the notations used in these three equations. The main method used to predict values of dietary variables of interest is also described fully in the appendix.

Statistical analyses and programming were conducted using STATA version 8.2 (412) and AMOS version 5.0 (441). We consider a structural model, in which *i* stands for individual and *j* for dietary variable *j* and T_{ij} (a latent variable) is the true value of dietary intake of nutrient or ratio of nutrients *j* for subject *i*. For that subject *i*, Q_{ij} is the value of dietary variable *j* derived from the food frequency questionnaire, T^*_{ij} is its value derived from the alloyed gold standard and M_{ij} is the value of instrumental biomarker for nutrient or nutrient ratio *j* (plasma cholesteryl ester level of fatty acids). In addition, N_{ij} is another biomarker (plasma phospholipids level of fatty acids):

$$Q_{ij} = \alpha_{0Q_j} + \alpha_{1Q_j} T_{ij} + \varepsilon_{Q_{ij}}$$

$$T^*_{ij} = \alpha_{0T^*_j} + \alpha_{1T^*_j} T_{ij} + \varepsilon_{T^*_{ij}}$$

$$M_{ij} = \alpha_{0M_j} + \alpha_{1M_j} T_{ij} + \varepsilon_{M_{ij}} \qquad \textbf{(4.3)}$$

$$N_{ij} = \alpha_{0N_j} + \alpha_{1N_j} T_{ij} + \varepsilon_{N_{ij}}$$

We impose the following constraints and identification conditions: $\sigma^2_T = \sigma^2_Q = \sigma^2_{T^*} = \sigma^2_M = 1$; $\alpha_{0Q} = \alpha_{0T^*} = \alpha_{0M} = \alpha_{0N} = 0$. All error terms ε were centered at zero. Hence, for each variable *j*, we attempted to estimate ten parameters with their standard errors (SE) namely: α_{1Q}; α_{1M}; α_{1N}; α_{1T^*}; $\sigma^2\varepsilon_Q$; $\sigma^2\varepsilon_{T^*}$; $\sigma^2\varepsilon_M$; $\sigma^2\varepsilon_N$; Corr(ε_N, ε_{T^*}); Corr(ε_N, ε_M) . The unconstrained model is over- identified with four degrees of freedom (d.f.): fourteen sample moments - ten free parameters. The model fit can be assessed as compared to a saturated model, using Root Mean Square Error of Approximation (RMSEA) with its 90 percent confidence interval (CI), as well as the χ^2 test. The criterion of a good fit as suggested by Hu and Bentler is RMSEA<0.06 and a non-significant χ^2 test (442). In cases where error variances were found to be negative (Heywood cases), alternative models were selected with either Corr(ε_N, ε_M) or Corr(ε_{T^*}, ε_N) are set to zero or both, leading to degrees of freedom of 5 and 6, respectively. In addition, the error variance was set to zero if needed along with the corresponding variable loading α which was set to 1, leading to even larger number of degrees of freedom (d.f.=8). However, if after these constraints, we failed to reject the null hypothesis that $\sigma^2\varepsilon = 0$, the model was deemed having an improper solution and was not presented (443). Q_{ij}, T^*_{ij}, M_{ij} and N_{ij} are entered into the model as z-scored variables.

This set of equations allowed us to estimate the attenuation factor :

λ_j = Cov(Q,T)/Var(Q) = $\dfrac{\alpha_{1Q_j}}{\alpha_{1Q_j}^2 + \sigma_{\varepsilon_{Q_j}}^2 / \sigma_T^2}$ = Corr(Q,T) with its approximate SE. In addition, the variance of measurement error $u_{ij} = Q_{ij} - T_{ij}$ was estimated for each variable j ($\sigma^2 u_j = \sigma^2 \varepsilon_{Qj}$), given that Q_j, T^*_j, M_j and N_j are linearly related to the true value T_j. An alternative method was used which allowed us to obtain covariances between errors in Q_j rather than assuming the matrix to be diagonal. Using the maximum-likelihood method of fitting the model, we computed a predicted score for T_{ij} through estimation of the factor score weights, using the regression method. These weights are computed by the following formula:

$$\mathbf{W_j} = \mathbf{B_j S_j^{-1}} \qquad \textbf{(4.4)}$$

Where, for each variable j: $\mathbf{W_j}$ is the matrix of regression weights; $\mathbf{S_j}$ is the matrix of covariances among the observed variables (Q, T^*, M and N); and $\mathbf{B_j}$ is the matrix of covariances between unobserved and observed variables (T *vs.* Q, T^*, M and N). Each factor score can be estimated as follows:

$$\hat{T}_{ij} = \hat{W}_{Qj} Q_{ij} + \hat{W}_{T^*j} T^*_{ij} + \hat{W}_{Mj} M_{ij} + \hat{W}_{Nj} N_{ij} \qquad \textbf{(4.5)}$$

The variance-covariance matrix of errors $\hat{u}_{ij} = Q_{ij} - \hat{T}_{ij}$ or $\hat{\Sigma}_{uu}$ was estimated for both absolute and ratio measures j.

E.2. Regression Calibration

Estimating the attenuation factor and the variance of the measurement error in an exposure variable or covariate constitutes the first step for measurement error adjustment using regression calibration (444). If a causal model **(4.6.1)** with a response variable Y contains one error-prone explanatory variable Q_j, correction for attenuation, assuming non-differential misclassification, consists simply of dividing the regression coefficient $\beta_{(naïve,\ j)}$ of that variable Q_j by λ_j **(4.6.2)**.

Variance estimates for the corrected regression coefficient of effect as well as 95 percent CI are estimated using **4.6.3** and **4.6.4**.

$$\mathrm{E}(Y|Q_j) = \hat{\beta}_0 + \hat{\beta}_{(\text{naïve, j})}Q_j \quad \textbf{(4.6.1)}$$

$$\hat{\beta}_{(\text{RC, j})} = \hat{\beta}_{(\text{naïve, j})} / \hat{\lambda}_{\text{j}} \quad \textbf{(4.6.2)}$$

$$\mathrm{Var}(\hat{\beta}_{(\text{RC, j})}) = \mathrm{Var}(\hat{\beta}_{(\text{naïve, j})})/\hat{\lambda}_j^2 + \hat{\beta}^2_{(\text{naïve, j})}\ \mathrm{Var}(\hat{\lambda}_{\text{j}})/\hat{\lambda}_j^4 \text{ and } \mathrm{SE}(\hat{\beta}_{(\text{RC, j})}) = \sqrt{\mathrm{Var}(\hat{\beta}_{(\text{RC,j})})} \quad \textbf{(4.6.3)}$$

$$95 \text{ percent CI } \beta_{(\text{RC, j})} \equiv \hat{\beta}_{(\text{RC, j})} \pm 1.96 \times \mathrm{SE}(\hat{\beta}_{(\text{RC, j})}) \quad \textbf{(4.6.4)}$$

For logistic regression, the assumptions made are linear homoscedastic regression of T on Q with a normally distributed error term and a rare disease requirement (444). Carroll and Stefanski (445) showed that the assumption of normality of f(T|Q) is not needed. Subsequently Kuha (446) introduced two key requirements for approximate unbiasedness of β_{RC}: **(i)** $\beta^2_1 * \sigma^2$ product is small; where $\sigma^2 = \mathrm{Var}\,(T|Q, Z_k)$; **(ii)** $\Pr(Y=1|T)$ is small and $f(T|Q)$ is normal.

In cases where more than one error-prone variable and several perfectly measured variables are introduced into a causal model **(4.7)**, the attenuation factor is no longer the parameter to be used for calibration.

$$\mathrm{E}(Y|Q_j, Z_k) = \hat{\beta}_0 + \sum_{j=1}^{\text{m}} \hat{\beta}_{\text{T,naïve},j}\ Q_j + \sum_{k=1}^{\text{n}} \hat{\beta}_{\text{Z,naïve},k} Z_k \quad \textbf{(4.7)}$$

As an alternative, regression calibration becomes reliant on the variance-covariance matrix of error in the measurement of different error-prone variables Q_j ($\hat{\Sigma}_{\text{uu}}$) as well as the variance-covariance matrices of the variables themselves. This relationship termed "method of moments" can be summarized as follows (447):

$$\begin{pmatrix} \hat{\beta}_{Z,RC} \\ \hat{\beta}_{T,RC} \end{pmatrix} = \begin{pmatrix} \Sigma_{ZZ} & \Sigma_{ZQ} \\ \Sigma_{QZ} & \Sigma_{QQ} - \hat{\Sigma}_{uu} \end{pmatrix}^{-1} \begin{pmatrix} \Sigma_{ZZ} & \Sigma_{ZQ} \\ \Sigma_{QZ} & \Sigma_{QQ} \end{pmatrix} \begin{pmatrix} \hat{\beta}_{Z,naive} \\ \hat{\beta}_{T,naive} \end{pmatrix} \quad \textbf{(4.8)}$$

The parameters that were presented include: Mean and SD for all continuous variables considered, proportions for categorical variables, factor loadings of T_j on T^*_j, M_j, and N_j, the attenuation factor $\hat{\lambda}_j$, and $\hat{\Sigma}_{uu}$ upon post-estimation of T_{ij} from the structural model. For several values of $\beta_{(\text{naïve, j})}$ and an associated $\text{Var}(\beta_{(\text{naïve, j})}) = 0.0025$, we estimated regression calibrated values $\beta_{(\text{RC, j})}$. Finally, P-values associated with Student's *t* and Pearson's χ^2 tests were computed to look for gender differentials in dietary, biomarker and other baseline measurements. Significance is set at an *a*-level of 0.05.

E.3. Simulation Extrapolation (SIMEX)

Simulation extrapolation or SIMEX is a procedure consisting of four main steps:

(i) Fitting the causal model to obtain the estimated coefficients $\beta_{\text{naïve}}$ and an estimate of the measurement error variance σ^2_u.

(ii) Generating random pseudo errors for a scale factor θ times the estimated error variance $\varepsilon \sim N(0, \theta\sigma^2_u)$. These pseudo errors are added to the original values of the error prone covariate. Fit the model to obtain $\beta\{\text{naïve}, \theta_j\}$. This is repeated r times to obtain mean coefficient vector $\beta\{\theta_j\} = (1/r)\Sigma\, \beta\{i, \theta_j\}$.

(iii) The previous step is repeated for j=1 …, k* scale factors, where typically we use θ = {.5, 1, 1.5, 2}, though individual researchers may choose a longer list of scale factors. Using the typical list of scale factors, we have k=5 estimated coefficient vectors since k*=4 for the list above, and we have the estimated coefficient vector form the initial step (k=k*+1).

(iv) For each regression coefficient β_m (m=1, …,p) in the model, we consider the estimated coefficient as a function of the scale factor θ_j for j=1, …k. Formally, we specify a function f( ) such that $\beta_m = f(\theta, \beta_m^{\{\theta\}})$. We estimate this relationship and then extrapolate back the final estimates $\beta_m = f(\theta_0 = -1, \beta_m^{\{\theta\}})$ (no measurement error). Researchers are free to choose the form of the function f( ), but we point out that there are relatively few – in this case 5 – observations available to estimate the parameters of f( ). The function f( ) used to model the relationship between the estimated coefficient and θ is called the *extrapolant function* (448). Although deciding which model to fit is a valid question when performing SIMEX, it has been shown that conservative estimates with a quadratic curve do improve over the naïve estimator without any correction. Investigators may also use model fitting techniques to decide which model to fit and then extrapolate with. Calculating the standard error of the SIMEX estimator requires 100 simulations on its own. With the ever increasing speed of computers, the necessary computing power is widely available (449).

E.4. Main Study Analysis

We carried out univariate analyses of predictor and outcome variables as well as covariates. For bivariate analyses of exposure and outcome, we computed means of predictor variables across outcome groups (0: no decline; 1: declined) and assessed statistical significance of differences using independent samples *t*-test at an alpha level of 0.05. We computed odds ratios of decline by each increase in exposure by 1 SD by conducting multivariate logistic regression analysis. Control for confounding was done using backward elimination and an overall change in estimate criterion of 5%. Covariates which changed the estimated effect of the exposure by more than 5% were retained in the final model.

Covariates considered as potential confounders were: baseline cognitive functioning, socio-demographics (age, sex, education, race), genetic factors (ApoE ε4 allele); behavioral factors (smoking, alcohol and caffeine consumption and physical activity) and nutritional factors (body mass index, caloric intake, other fatty acids, intake of antioxidants and vitamins B_6, B_{12} and folate, other dietary fatty acids). Hypertension was considered as a potential effect modifier. Likelihood ratio tests were used to assess statistical significance of interaction between exposure and hypertensive status at a type I error level of 0.20, after obtaining the final parsimonious model. All other covariates were deemed as potential intermediates and hence were not included in the multivariate models. The multivariate models can be summarized by equations **(4.9.1)** and **(4.9.2)**:

$$\text{Logit}[\Pr(Y=1 \mid Q, Z)] = \beta_0 + \beta_1 Q_1 + \Sigma\beta_{2i} Q_{2i} + \Sigma\beta_j Z_j \qquad \textbf{(4.9.1)}$$

$$\text{Logit}[\Pr(Y=1 \mid Q, Z, H)] = \beta_0 + \beta_1 Q_1 + \Sigma\beta_{2i} Q_{2i} + \beta_3 H + \Sigma\beta_j Z_j + \gamma Q_1 \times H \qquad \textbf{(4.9.2)}$$

Where Logit is the $\text{Log}_e[\Pr/(1-\Pr)]$, Y is the binary outcome of cognitive decline, Q_1 is the main exposure of interest as derived from the FFQ, Q_{2i} are the other fatty acids that might act as confounders, Z_j is a vector of potential confounders that are assumed to be perfectly measured, and H is the effect modifier "hypertensive status" also assumed to be perfectly measured. The same process was used with the plasma exposures in cholesteryl esters and phospholipids. To correct for measurement error in dietary exposure, a sensitivity analysis was conducted for models **(1)** and **(2)** whereby regression calibration and simulation extrapolation were applied to the final parsimonious models for each outcome/exposure pair (449, 450). Statistical analyses were conducted using STATA ver. 8.2 (412).

E.5. Additional Sub-group Analyses

Additional sub-group analysis was conducted similar to above. However, the analysis was restricted to the study population with plasma fatty acids available and measured at baseline (i.e. Minneapolis whites).

Plasma cholesteryl ester and phospholipid long chain ω-3 fatty acids were interacted with the following oxidative-stress inducing conditions: Apo E ε4 allele, hypertension, stroke/TIAs, type II diabetes mellitus, dyslipidemia, hypercoagulable profile, depression and poor pulmonary function. All these were measured using the definitions in **Table 4.6**. A cutoff point for depression was chosen based on quintile distribution and the uppermost quintile was compared to the rest of the population. In addition, all fatty acid groups (e.g. saturated, mono-unsaturated, poly-unsaturated) as well as selected individual fatty acids (e.g. palimitic, stearic, oleic, linoleic) were studied in relation to global cognitive decline.

Chapter 5

RESULTS

A. Validation of essential fatty acid intake from a semi-quantitative food frequency questionnaire using an alloyed gold standard and instrumental biomarkers

The use of food frequency questionnaires (FFQ) in epidemiological studies has been plagued by significant amounts of measurement error. Since error-prone exposures may cause bias in relative risk estimates, it is crucial to assess validity of intake measures to enhance interpretation of diet-disease associations, improving their translation into dietary recommendations. Regression calibration is a common method of adjusting relative risk for error in exposure. Fatty acid intake from a semi-quantitative FFQ administered in ARIC study at baseline (1987-89) was considered. Among middle-aged adults, 2,834 white subjects residing in Minneapolis had additional data on plasma phospholipids and cholesteryl ester fatty acids concentrations. This is the first use of structural models to estimate error variance-covariance matrix and attenuation factors of fatty acid exposures. These parameters constitute primary inputs into a regression calibration analysis. While FFQ is our test measure, validation included as references, an alloyed gold standard derived from biomarkers and empirical equations model and two other instrumental biomarkers required to be linearly correlated with true intake. Findings from our study may be applied to adjust measurement error in research using this FFQ on similar populations. Biomarkers must be unbiased estimates of truth for the methods of this paper to be applicable.

A.1. Introduction

The complexity of dietary behavior has been contrasted with the simplicity of dietary assessment tools. Consequently, measuring usual intake of nutrients with semi-quantitative food frequency questionnaires (FFQ) in epidemiological studies as an exposure to common chronic diseases has been plagued by random and systematic errors (429). Since systematic errors generally cause bias in relative risk estimates derived from multivariate models, it is crucial to assess validity of intake measures, in order to enhance the interpretation of estimated diet-disease associations and to improve their translation into dietary recommendations (421, 430). One cost of measurement error in multivariate analyses is loss of statistical power. Assuming random error that is independent of true value of exposure and outcome *Y*, regression calibration is a technique which corrects for biases in primary regression models in situations where exposure variables are measured with error. The existence of a validation sub-study, where accurate and crude measurement methods are related by a second regression analysis, is assumed (431). Food frequency questionnaires (FFQ) have been historically validated against another dietary assessment method assumed to be more accurate such as multiple 24-hour recalls or food records of food intake (432-434). The problem attending this approach is that the same factors that affect these reference methods (*R*) may also affect the FFQ-based assessments (*Q*). This problem would make it impossible to presume independent random errors in the two methods, which in turn leads to over-estimation of the correlation between the reference method and the FFQ (435). Hence, to consider a method a gold standard, it should ideally be an accurate depiction of the truth and any error associated with it should be independent of errors that the test measure itself carries. Such restriction on measurement error associations makes biomarkers a desirable target for validation studies.

The present study aims at validating an FFQ for the estimation of essential fatty acid intake and biologically plausible combinations and ratios of these nutrients. The study utilizes two reference measures: (i) an alloyed gold standard (*T**) based on a biomarker of specific fatty acid levels in plasma phospholipids and empirically derived biochemical equations that estimate true dietary intake (381-383). This alloyed gold standard takes the biochemical approach to validation (ii) Two instrumental biomarkers for the level of fatty acids in the cholesteryl ester (*M*) and phospholipids (*N*) fractions of plasma which were previously shown to be linearly related to dietary intake as assessed by more reliable reference methods such as multiple 24-hour recalls, food records or diet history (373-375, 436-440). These instrumental biomarkers take an epidemiological approach to validation. Findings from our study and its overall methodology may be used in subsequent analyses to adjust for measurement error in primary regression linking intake of essential fatty acids and their biologically plausible ratios with disease outcomes.

A.2. Methods

A.2.1. Study Population

The Atherosclerosis Risk in Communities (ARIC) is a prospective study conducted between 1987 and 1998 to investigate the etiology of atherosclerosis and its clinical sequelae and variation in cardiovascular risk factors, medical care, and disease by race, sex, place, and time in each of four US communities, namely Forsyth County, North Carolina, Jackson, Mississippi, suburbs of Minneapolis, Minnesota, and Washington County, Maryland. Using probability sampling, ARIC included 15,792 men and women between the ages of 45 and 65 years at baseline (403).

We restricted our population to 2,834 white men and women aged 50-65 years at baseline, residing in the Minneapolis metropolitan area, since this selective group has complete data on dietary and plasma levels of fatty acids and the age group is of particular interest to study the effect of exposures among middle-aged and older adults.

A.2.2. Diet Assessment

Usual dietary intake was estimated using an interviewer-administered semi-quantitative FFQ modified from a 61-item questionnaire previously developed and validated by Willet and colleagues against multiple food records among a sub-sample of the Nurse's Health Study cohort (416). Subjects were asked how often, on average, they had consumed certain foods in portions of a specified size during the preceding year. There were nine possible responses, ranging from "almost never" to "more than six times per day." Daily intake of nutrients was calculated by multiplying the nutrient content of each food in the portion specified by the frequency of daily consumption and summing the results. The nutrient content of each food was obtained from the Harvard nutrient data base for which the primary source was the Department of Agriculture handbook(417). Dietary intake of essential fatty acids and their elongated and desaturated products can be expressed as percent of total energy intake and grouped under four main categories: (*3P*) ω -3 UFAs: 18:3+18:4ω-3; (*6P*) ω -6 UFAs: 18:2+{18:3ω-6}; (*3H*) ω-3HUFAs: 20:5+22:5+22:6ω-3 and (*6H*) ω-6 HUFAs: {20:3}+20:4+{22:4+22:5} ω-6. Fatty acids between braces were imputed as very small numbers (0.003 percent for 18:3 ω-6 and 0.002 percent for 20:3+22:4+22:5 ω-6 as estimated from the literature) since they could not be directly analyzed from the FFQ.

A.2.3. Alloyed gold standard and instrumental biomarkers

Twelve-hour fasting blood was collected according to the ARIC study wide protocol. The Minneapolis field center conducted fatty acid analysis in plasma phospholipid and cholesteryl ester fractions for visit 1 blood specimens among the white segment of the study population in that center. The procedure is described in detail elsewhere (422). The identity of 28 fatty acid peaks were revealed by gas chromatography by comparing each peak's retention time to the retention times of fatty acids in synthetic standards of known compositions. The relative amount of each fatty acid (as a percent of all fatty acids) could be calculated by integrating the area under the peak and dividing the result by the total area for all fatty acids and multiplying by 100. To minimize transcription errors, data from the chromatogram was transferred electronically to a computer for analysis. Two instrumental biomarkers, consisting of the plasma phospholipids and cholesteryl ester level of fatty acids in each of the groups described above, were used to assess measurement error in the FFQ.

While most epidemiological studies assume a linear relationship between dietary fatty acid intake and different biomarkers, the metabolic interactions of these fatty acids upon assimilation into different compartments suggest otherwise. Biochemical studies in the area of lipid research conducted by Lands and colleagues (381-383) suggest that the mixture of 20-C and 22-C highly unsaturated fatty acids (HUFAs) that are maintained in phospholipids of human plasma is related to the dietary intake of the four groups of fatty acids that were described earlier, expressed as percent of total energy intake. This relationship was empirically derived and is hyperbolic in nature among both humans and laboratory rats. The derived empirical equations were successful at reflecting the general metabolic selectivities that maintain fatty-acid composition in plasma phospholipids.

The authors clearly stated that these equations can be used in reverse to estimate dietary intake of the ω-3 and ω-6 fatty acids by using the composition of the fatty acids that had been maintained in plasma phospholipids. The following competitive hyperbolic relationship reflecting saturable rate-limiting biochemical processes was used to derive these equations:

$$\text{Response} = \frac{V_{max}}{1+\frac{K_m}{en\%S}\left(1+\frac{en\%I}{K_i}\right)} \qquad \textbf{(5.1)}$$

Where V_{max} in this case is 100, K_m and K_i are constants that are closely related to the type of dietary fatty acid (*S* or *I*) in question. In particular, the following three empirically derived equations (**5.2.1** through **5.2.3**) were used in our analyses:

$$pfh_6 = \left(1+\frac{C_6}{T_{6p}}\left(1+\frac{T_{3p}}{C_3}+\frac{T_o}{C_o}+\frac{T_{6p}}{K_S}\right)\right)^{-1} 100 \qquad \textbf{(5.2.1)}$$

$$pfh_3 = \left(1+\frac{C_3}{T_{3p}}\left(1+\frac{T_{6p}}{C_6}+\frac{T_o}{C_o}+\frac{T_{3p}}{K_S}\right)\right)^{-1} 100 \qquad \textbf{(5.2.2)}$$

$$pfh_6 = \left(1+\frac{PC_6}{T_{6p}}\left(1+\frac{T_{3p}}{PC_3}+\frac{T_{3p}}{HI_3}+\frac{T_o}{C_o}+\frac{T_{6p}}{K_S}\right)\right)^{-1} 100 + \left(1+\frac{HC_6}{T_{6H}}\left(1+\frac{T_{3H}}{HC_3}\right)\right)^{-1} 100 \qquad \textbf{(5.2.3)}$$

Appendix A Table A.1. shows definitions and values of each of the notations used in these three equations. The main method used to predict values of dietary variables of interest is also described fully in the appendix.

A.2.4. Statistical analysis

Statistical analyses and programming were conducted using STATA version 8.2 (412) and AMOS version 5.0 (441). We consider a structural model, in which *i* stands for individual and *j* for dietary variable *j* and T_{ij} (a latent variable) is the true value of dietary intake of nutrient or ratio of nutrients *j* for subject *i*.

For that subject *i*, Q_{ij} is the value of dietary variable *j* derived from the food frequency questionnaire, T^*_{ij} is its value derived from the alloyed gold standard and M_{ij} is the value of instrumental biomarker for nutrient or nutrient ratio *j* (plasma cholesteryl ester level of fatty acids). In addition, N_{ij} is another biomarker (plasma phospholipids level of fatty acids). All measured quantities were standardized into z-scores. Latent variable *T* was constrained to have a mean of zero and a variance of 1.

$$Q_{ij} = \alpha_{0Q_j} + \alpha_{1Q_j} T_{ij} + \varepsilon_{Q_{ij}}$$

$$T^*_{ij} = \alpha_{0T^*_j} + \alpha_{1T^*_j} T_{ij} + \varepsilon_{T^*_{ij}}$$

$$M_{ij} = \alpha_{0M_j} + \alpha_{1M_j} T_{ij} + \varepsilon_{M_{ij}} \qquad \textbf{(5.3)}$$

$$N_{ij} = \alpha_{0N_j} + \alpha_{1N_j} T_{ij} + \varepsilon_{N_{ij}}$$

We impose the following constraints and identification conditions: $\sigma^2_T = \sigma^2_Q = \sigma^2_{T^*} = \sigma^2_M = 1$; $\alpha_{0Q} = \alpha_{0T^*} = \alpha_{0M} = \alpha_{0N} = 0$. All error terms ε were centered at zero. Hence, for each variable *j*, we attempted to estimate ten parameters with their standard errors (SE) namely: α_{1Q}; α_{1M}; α_{1N}; α_{1T^*}; $\sigma^2\varepsilon_Q$; $\sigma^2\varepsilon_{T^*}$; $\sigma^2\varepsilon_M$; $\sigma^2\varepsilon_N$; Corr(ε_N, ε_{T^*}); Corr(ε_N, ε_M) . The unconstrained model is over- identified with four degrees of freedom (d.f.): fourteen sample moments - ten free parameters. The model fit can be assessed as compared to a saturated model, using Root Mean Square Error of Approximation (RMSEA) with its 90 percent confidence interval (CI), as well as the χ^2 test. The criterion of a good fit as suggested by Hu and Bentler is RMSEA<0.06 and a non-significant χ^2 test (442). In cases where error variances were found to be negative (Heywood cases), alternative models were selected with either Corr(ε_N, ε_M) or Corr(ε_{T^*}, ε_N) are set to zero or both, leading to degrees of freedom of 5 and 6, respectively.

In addition, the error variance was set to zero if needed along with the corresponding variable loading α which was set to 1, leading to even larger number of degrees of freedom (d.f.=8). However, if after these constraints, we failed to reject the null hypothesis that $\sigma^2\varepsilon = 0$, the model was deemed having an improper solution and was not presented (443). Q_{ij}, T^*_{ij}, M_{ij} and N_{ij} are entered into the model as z-scored variables.

This set of equations allowed us to estimate the attenuation factor :

$\lambda_j = \text{Cov}(Q,T)/\text{Var}(Q) = \dfrac{\alpha_{1Q_j}}{\alpha^2_{1Q_j} + \sigma^2_{\varepsilon_{Q_j}} / \sigma^2_T} = \text{Corr}(Q,T)$ with its approximate SE. In addition, the variance of measurement error $u_{ij} = Q_{ij} - T_{ij}$ was estimated for each variable j ($\sigma^2 u_j = \sigma^2 \varepsilon_{Qj}$), given that Q_j, T^*_j, M_j and N_j are linearly related to the true value T_j. An alternative method was used which allowed us to obtain covariances between errors in Q_j rather than assuming the matrix to be diagonal. Using the maximum-likelihood method of fitting the model, we computed a predicted score for T_{ij} through estimation of the factor score weights, using the regression method. These weights are computed by the following formula:

$$\mathbf{W_j} = \mathbf{B_j S_j^{-1}} \qquad \textbf{(5.4)}$$

Where, for each variable j: $\mathbf{W_j}$ is the matrix of regression weights; $\mathbf{S_j}$ is the matrix of covariances among the observed variables (Q, T^*, M and N); and $\mathbf{B_j}$ is the matrix of covariances between unobserved and observed variables (T *vs.* Q, T^*, M and N). Each factor score can be estimated as follows:

$$\hat{T}_{ij} = \hat{W}_{Qj} Q_{ij} + \hat{W}_{T^*j} T^*_{ij} + \hat{W}_{Mj} M_{ij} + \hat{W}_{Nj} N_{ij} \qquad \textbf{(5.5)}$$

The variance-covariance matrix of errors $\hat{u}_{ij} = Q_{ij} - \hat{T}_{ij}$ or $\hat{\Sigma}_{uu}$, as estimated for both absolute and ratio measures, is presented for untransformed values of j.

Estimating the attenuation factor and the variance of the measurement error in an exposure variable or covariate constitutes the first step for measurement error adjustment using regression calibration. According to Rosner and colleagues (444), if a primary regression model **(5.6.1)** with a response variable *Y* contains one error-prone explanatory variable Q_j, correction for attenuation, assuming non-differential misclassification, consists simply of dividing the regression coefficient $\beta_{(naïve,\,j)}$ of that variable Q_j by λ_j **(5.6.2)**. Variance estimates for the corrected regression coefficient of effect as well as 95 percent CI are estimated using **5.6.3** and **5.6.4**.

$$E(Y|Q_j) = \hat{\beta}_0 + \hat{\beta}_{(naïve,\,j)}Q_j \qquad \textbf{(5.6.1)}$$

$$\hat{\beta}_{(RC,\,j)} = \hat{\beta}_{(naïve,\,j)} / \hat{\lambda}_j \qquad \textbf{(5.6.2)}$$

$$Var(\hat{\beta}_{(RC,\,j)}) = Var(\hat{\beta}_{(naïve,\,j)})/\hat{\lambda}_j^2 + \hat{\beta}^2_{(naïve,\,j)}\, Var(\hat{\lambda}_j)/\hat{\lambda}_j^4 \text{ and } SE(\hat{\beta}_{(RC,\,j)}) = \sqrt{Var(\hat{\beta}_{(RC,j)})} \qquad \textbf{(5.6.3)}$$

$$95 \text{ percent CI } \beta_{(RC,\,j)} \equiv \hat{\beta}_{(RC,\,j)} \pm 1.96 \times SE(\hat{\beta}_{(RC,\,j)}) \qquad \textbf{(5.6.4)}$$

For logistic regression, the assumptions made are linear homoscedastic regression of T on Q with a normally distributed error term and a rare disease requirement (444). Carroll and Stefanski (445) showed that the assumption of normality of f(T|Q) is not needed. Subsequently Kuha (446) introduced two key requirements for approximate unbiasedness of β_{RC}: **(i)** $\beta^2_1 * \sigma^2$ product is small; where $\sigma^2 = Var\,(T|Q, Z_k)$; **(ii)** $Pr(Y=1|T)$ is small and $f(T|Q)$ is normal.

In cases where more than one error-prone variable and several perfectly measured variables are introduced into a primary regression model **(5.7)**, the attenuation factor is no longer the parameter to be used for calibration.

$$E(Y|Q_j, Z_k) = \hat{\beta}_0 + \sum_{j=1}^{m} \hat{\beta}_{T,naïve,j}\, Q_j + \sum_{k=1}^{n} \hat{\beta}_{Z,naïve,k} Z_k \qquad \textbf{(5.7)}$$

As an alternative, regression calibration becomes reliant on the variance-covariance matrix of error in the measurement of different error-prone variables Q_j ($\hat{\Sigma}_{uu}$) as well as the variance-covariance matrices of the variables themselves. This relationship termed "method of moments" can be summarized as follows (447):

$$\begin{pmatrix} \hat{\beta}_{Z,RC} \\ \hat{\beta}_{T,RC} \end{pmatrix} = \begin{pmatrix} \Sigma_{ZZ} & \Sigma_{ZQ} \\ \Sigma_{QZ} & \Sigma_{QQ} - \hat{\Sigma}_{uu} \end{pmatrix}^{-1} \begin{pmatrix} \Sigma_{ZZ} & \Sigma_{ZQ} \\ \Sigma_{QZ} & \Sigma_{QQ} \end{pmatrix} \begin{pmatrix} \hat{\beta}_{Z,naive} \\ \hat{\beta}_{T,naive} \end{pmatrix} \quad \textbf{(5.8)}$$

Alternatively, one can use simulation extrapolation (SIMEX) which also relies on the method of moments with an estimate of $\hat{\Sigma}_{uu}$ or replicate measures of the error-prone variable. This method is described in detail in (449). The parameters that were presented include: Mean and SD for all continuous variables considered, proportions for categorical variables, factor loadings of T_j on T^*_j, M_j, and N_j, the attenuation factor $\hat{\lambda}_j$, and $\hat{\Sigma}_{uu}$ upon post-estimation of T_{ij} from the structural model. For several values of $\beta_{(\text{naïve, j})}$ and an associated Var($\beta_{(\text{naïve, j})}$) = 0.0025, we estimated regression calibrated values $\beta_{(RC, j)}$. Finally, *p*-values associated with independent samples *t* and Pearson's χ^2 tests were computed to look for gender differentials in dietary, biomarker and other baseline measurements. Null hypothesis is that of no difference across genders and significance is set at an *a*-level of 0.05.

A.3. Results

The cohort under study consisted of 2,834 white older adults aged between 50 and 65 years at baseline (1987-89) residing in the suburbs of Minneapolis, 1,404 of whom were women. While age was almost equally distributed between the 5-year brackets among men, women were more concentrated in the age group 50-54 (41.5 percent). In terms of education, a wide gender gap was noted whereby 66 percent of men belonged to the uppermost category of attainment as compared to only 45.3 percent among women.

Body mass index was on average higher among men and overall, 24.1 percent of the population was obese. Over half of the men were former smokers as compared to only around 30 percent of women. Alcohol and total energy intake were more elevated among men ($p<0.001$). In contrast, Vitamins A and E intake was higher among women, while no significant difference between genders was shown for Vitamin C consumption (**Table 5.1.1**).

Table 5.1.2 presents means and standard deviations of observed fatty acid intake from FFQ (Q) and alloyed gold standard measure based on plasma phospholipid biomarkers and empirical equations models (T^*) as well as instrumental biomarker level in the cholesteryl ester and phospholipids fraction of plasma corresponding to each intake variable j (M and N). Comparing mean Q to mean T^* levels, we find that in general, Q is an underestimate of T^* particularly for absolute measures of fatty acid intake. For ratio of ω-3 to ω-6 intake, underestimation was found only for $j = 3H/6H$, while $3P/6P$ and $3/6$ were slightly overestimated. Statistically significant gender differentials were noted for most Q, M and N variables, but only one T^* variable (T^*_{3p}).

To prove that the method used was able to provide combinations of fatty acids that would improve the fit of observed values of *pfh3* and *pfh6* to the empirical equations model, we entered T^* followed by Q combinations of dietary intake values into equations **(5.2.1)** through **(5.2.3)** and estimated the predicted values of *pfh6* and *pfh3* accordingly. **Figure 5.1.1** shows a scatter plot of predicted values of these biomarkers as compared to their observed values. Using equation **(5.2.1)** which predicted *pfh6* based on combinations of $3P$, $6P$ and O fatty acid intake, the correlation between observed and predicted when entering Q in contrast to T^* combinations was increased from 0.25 to 0.87. Similarly, and because of the methodology used, a perfect fit was produced for equation **(5.2.2)** which predicted *pfh3* with the T^* combination (r=1.00) as compared to a flat slope with the Q combinations (r=0.19).

Finally, for the third empirical equation **(5.2.3)** which again predicted *pfh6* but this time by adding the effects of *3H* and *6H* fatty acids, the correlation between predicted and observed was increased from as low as r=0.04 for the Q combination to as high as r=0.37 for the *T** combination. Hence, overall, the method was able to improve the fit of all three equations and produce combinations of fatty acid intake that are in line with the empirical equations model and the levels of biomarkers observed. It is therefore possible to consider T_j^* as alloyed gold standards against which one can compare to the Q_j dietary intake variables derived from the semi-quantitative FFQ.

Table 5.1.3 presents the results of the structural model with focus on the free parameters that were estimated, including factor loadings with their SE and the error variances. The results are shown for untransformed values of variables *j*. While the biomarkers *M* and *N* loaded heavily on all measures of intake, the alloyed gold standard *T** was mostly influencing the distribution of ratio variables of intake (i.e. *3P/6P*, *3H/6H* and *3/6*) as well as intake of long chain ω-3 fatty acids (*3H*). The attenuation factor ($\lambda_j = \alpha_{1Q}$) ranged between 0.056 for *6H* and 0.494 for *3H/6H*. Model fit was acceptable for all *j* variables based on χ^2 test and RMSEA statistics.

Using estimates of factor score weights $\hat{W}_{Qj}, \hat{W}_{T^*j}, \hat{W}_{Mj}$ and $\hat{W}_{Nj}$, factor scores were reconstructed from the original measured variable scores. Findings from **Table 5.1.4** indicated that error in Q_j was most pronounced for variable *6P*, *6H, 6* and *3+6* ($\hat{\sigma}_u^2 > 1.5$) which is in line with findings from Table 3. In addition, errors were correlated between specific sets of variables, indicating that if these variables were entered simultaneously in a primary regression model, the measurement error variance-covariance matrix should take into consideration these correlations.

However, a closer look at these correlations show that primary regression models that are usually fit would not include these variables simultaneously (e.g. *3* and *3P* at the same time) hence reducing the danger of multicollinearity. The variance-covariance matrix can be used to adjust for measurement error in error-prone variables that are entered into a multivariate primary regression model.

A.4.Discussion

This is one of the very few attempts to estimate an error variance-covariance matrix ($\hat{\Sigma}_{uu}$) that can be used subsequently by other researchers for the purpose of correcting for measurement error in multivariate generalized linear models. Estimates of attenuation factors (λ_i) which can be used mainly in bivariate generalized linear models were also reported. The approach used was similar to previous research (429, 451, 452). While this article focused on regression calibration, other measurement error models utilize $\hat{\Sigma}_{uu}$, including simulation extrapolation (SIMEX), methods with instrumental variables and maximum-likelihood methods(447). Health outcomes that have traditionally been of interest in relation to essential fatty acids and the balance between them include coronary heart disease (324, 325), stroke (327, 328), type II diabetes (341), breast and prostate cancer (453, 454), depression (351, 352), cognitive functioning (137, 141, 388, 389), hypercoagulable profile (332, 336) and COPD (422). While the transportability of this estimate of error variance may be limited to older adults participating in the ARIC study, it is also possible to utilize it for other studies using the same FFQ instrument and having participants with similar characteristics. One major implication to measurement error, as stated earlier, is loss of statistical power to detect an exposure-disease association.

In fact, the sample size required to detect a specific risk ratio (e.g. RR=2) is inflated proportionally to the inverse squared attenuation factor. For instance, if the true λ_j was 0.2, the sample size, calculated by assuming that λ_j is equal to 0.4, should be multiplied by $0.4^2/0.2^2 = 4$ to achieve nominal power (455) . Table A.2. (Appendix A) presents a hypothetical sensitivity analysis where naïve estimates of regression coefficients from a disease model are calibrated using results from Table 3 and the method of regression calibration as outlined earlier. We assumed that standard errors of naïve estimates were 0.05. Using a set of equations, we estimated regression calibrated coefficients $\beta_{(RC,\ j)}$ and the corresponding SE for each variable *j*. For instance, for *3H* and for a logistic regression model of disease risk, the results would indicate that a 1 SD increase in *3H* would increase the odds of a disease by 63 percent rather than 22 percent after calibration. For a protective effect of *3H*, this corresponds to attenuation for the odds ratio (OR) from 0.61 to 0.82. However, since SE of the regression coefficient was increased from 0.050 to 0.103, the 95 percent CI for OR were wider after calibration. It is worth noting that because latent variable T (true intake of fatty acids as % of energy intake) is on a z-score standardized scale, the use of the attenuation factor and error variance covariance would lead to a calibrated standardized regression model. For a logistic model, an odds ratio is interpreted as increase or decrease in odds of disease with every SD increase in the continuous z-scored exposure.

Some of the main limitations of this study include the lack of a reference method that is known to be more reliable than FFQs in the ARIC study (e.g. multiple 24-hour recalls or food records). However, because of correlated errors between self-report methods, the use of biomarkers has often been cited as a more adequate means to assess the extent of measurement error in a test instrument.

Another drawback is the fact that plasma levels of fatty acids in both fractions studied constitute a short-term measure of intake although they have been shown to correlate well with long-term intake (9). In addition, the lack of certainty as to the nature of the relationship between the biomarkers considered and the intake variables and the potential interaction of these dietary exposures with other nutritional, environmental and genetic factors constitutes a major challenge for interpretation. For this reason, and using structural equations modeling, estimation of measurement error in FFQ derived nutrients took into consideration two approaches, by including an alloyed gold standard with assumed hyperbolic relationship with biomarkers in plasma phospholipids (a biochemical approach) and two instrumental biomarkers with assumed linear relationship with intake (an epidemiological approach). Finally, although there has been evidence of correlation between intake of fatty acids and their levels in the substrates considered in our study, such a correlation does not necessarily render these biomarkers an adequate reflection of long-term fatty acid intake. In fact, the only substrate that has been shown to work as a gold standard is adipose tissue. However, because of the elevated cost and invasiveness of the procedure, studies using adipose tissue fatty acid concentration as an intake biomarker were often of limited sample size and hence correlations obtained had insufficient levels of precision (377, 379). Another potentially adequate biomarker that was often used to validate medium-term intake of fatty acids is erythrocyte membrane concentration (369, 378). Future endeavors to correct for error should make use of structural equations modeling and include as many instrumental biomarkers as is available along with other self-reported or biomarker-based reference methods of dietary assessment. However, the choice of biomarkers and interpretation of their variability must be made as to account for biochemical and physiological interactions between dietary, environmental and genetic factors.

Moreover, one must be cautious of coupled errors between biological markers and must take into account these correlations when specifying the structural model. Finally, because structural equations modeling makes a strong assumption about joint multivariate normality, often not present, it is crucial for future studies to use newly developed methodologies which appear to be more flexible in many ways (456).

TABLE 5.1.1
Baseline characteristics of the study population of MN whites aged 50+ at visit 1 by gender; ARIC (1987-89)

	Men (N= 1,430)		Women (N= 1,404)		Total sample (N=2,834)	
Age(years)						
50-54	33.9*		41.5		37.7	
55-59	34.5		32.1		33.3	
60+	31.5		26.4		29.0	
Mean (SD†)	56.9*	(4.3)	56.1	(4.2)	56.5	(4.3)
Education						
Less than High school	8.5*		6.4		7.4	
High school graduate	25.5		48.2		36.8	
More than High school	66.0		45.3		55.8	
Body Mass Index (kg. per m^2)						
<25.00	22.7*		45.0		33.7	
25.0-29.9	51.6		32.6		42.2	
≥30	25.8		22.4		24.1	
Mean (SD)	27.8*	(3.8)	26.6	(5.1)	27.2	(4.5)
Smoking status						
Current smoker	20.2*		22.5		21.4	
Former smoker	52.7		29.9		41.4	
Never smoked	27.1		47.5		37.2	
Alcohol (g/d)	12.2*	(18.0)	4.7	(8.1)	8.5	(14.5)
Energy intake (Kcal/day)	1684*	(589)	1473	(511)	1579	(562)
Vitamin A (1000 IUs/day)	8.3*	(7.3)	9.0	(6.2)	8.6	(6.8)
Vitamin C (mg/day)	110.7	(72.4)	113.7	(69.1)	111.9	(70.8)
Vitamin E (mg/day)	4.8*	(3.4)	4.4	(2.4)	4.6	(2.9)

*p<0.05 for null hypothesis that mean or proportion among men is equal to mean or proportion among women.
† SD, standard deviation

TABLE 5.1.2
Fatty acid intake measures (Q, T*) and biomarker levels (M, N); ARIC (1987-89)

	Men		Women		Total Sample	
	Mean	(SD‡)	Mean	(SD)	Mean	(SD)
FFQ fatty acid intake (Q)†						
Q_{6p}	4.767*	(1.544)	4.508	(1.572)	4.638	(1.563)
Q_{3p}	0.413	(0.090)	0.410	(0.091)	0.412	(0.091)
Q_{6H}	0.075	(0.027)	0.074	(0.027)	0.074	(0.027)
Q_{3H}	0.135*	(0.137)	0.174	(0.167)	0.154	(0.154)
Q_6	4.841*	(1.546)	4.582	(1.574)	4.713	(1.565)
Q_3	0.531*	(0.146)	0.562	(0.177)	0.546	(0.163)
Q_{3+6}	5.390*	(1.545)	5.166	(1.571)	5.279	(1.561)
Q_{3p}/Q_{6p}	0.095*	(0.036)	0.102	(0.043)	0.098	(0.039)
Q_{3H}/Q_{6H}	1.791*	(1.634)	2.252	(1.754)	2.019	(1.710)
Q_3/Q_6	0.121*	(0.057)	0.138	(0.070)	0.130	(0.064)
Alloyed Gold Standard (T*)†						
T^*_{6p}	10.030	(3.543)	9.915	(3.505)	9.973	(3.524)
T^*_{3p}	0.571*	(0.227)	0.552	(0.225)	0.562	(0.226)
T^*_{6H}	0.233	(0.121)	0.228	(0.124)	0.231	(0.122)
T^*_{3H}	0.544	(0.270)	0.539	(0.275)	0.542	(0.272)
T^*_6	10.263	(3.507)	10.143	(3.469)	10.204	(3.488)
T^*_3	1.115	(0.383)	1.092	(0.382)	1.104	(0.383)
T^*_{3+6}	11.379	(3.758)	11.235	(3.719)	11.308	(3.739)
T^*_{3p}/T^*_{6p}	0.058	(0.016)	0.057	(0.021)	0.058	(0.019)
T^*_{3H}/T^*_{6H}	2.557	(1.092)	2.605	(1.152)	2.581	(1.123)
T^*_3/T^*_6	0.115	(0.041)	0.114	(0.046)	0.115	(0.043)
Instrumental Biomarker #1 (M)†						
M_{6p}	54.930	(4.460)	55.114	(4.817)	55.021	(4.641)
M_{3p}	0.388*	(0.099)	0.434	(0.108)	0.411	(0.106)
M_{6H}	8.947*	(1.657)	9.226	(1.820)	9.085	(1.745)
M_{3H}	0.977*	(0.391)	1.026	(0.435)	1.001	(0.414)
M_6	62.895*	(4.029)	63.266	(4.372)	62.079	(4.206)
M_3	1.365*	(0.422)	1.460	(0.464)	1.412	(0.446)
M_{3+6}	65.242*	(3.796)	65.800	(4.100)	65.518	(3.958)
M_{3p}/M_{6p}	0.007	(0.002)	0.008	(0.002)	0.007	(0.002)
M_{3H}/M_{6H}	0.111	(0.046)	0.113	(0.055)	0.112	(0.051)
M_3/M_6	0.022*	(0.007)	0.023	(0.008)	0.022	(0.008)
Instrumental Biomarker #2 (N)†						
N_{6p}	22.092	(2.576)	21.900	(2.737)	21.997	(2.658)
N_{3p}	0.130*	(0.044)	0.154	(0.051)	0.142	(0.049)
N_{6H}	15.715	(2.055)	15.857	(2.196)	15.785	(2.127)
N_{3H}	3.340*	(1.085)	3.488	(1.055)	3.413	(1.072)
N_6	37.700	(1.790)	37.646	(1.897)	37.673	(1.843)
N_3	3.469*	(1.085)	3.642	(1.053)	3.555	(1.073)
N_{3+6}	41.276	(0.046)	41.400	(0.049)	41.338	(1.775)
N_{3p}/N_{6p}	0.006*	(0.002)	0.007	(0.002)	0.006	(0.002)
N_{3H}/N_{6H}	0.217*	(0.085)	0.224	(0.083)	0.220	(0.084)
N_3/N_6	0.093*	(0.032)	0.097	(0.032)	0.095	(0.032)

*$p < 0.05$ for null hypothesis that mean among men is equal to mean among women.
† Units: Q is the test measure derived from food frequency questionnaire and T* is the alloyed gold standard derived from a set of biomarkers and empirical equations. Both are expressed as percent of total energy intake; M and N are instrumental biomarkers required to be linearly related to true dietary intake. They are expressed as percent of total fatty acid concentration in substrate. The substrate for M is plasma cholesteryl esters and for N is plasma phospholipids. ‡SD, Standard Deviation. § Subscript notations are described in the materials and methods section, under "dietary assessment".

TABLE 5.1.3.
Structural equations model: Factor loading estimates with standard errors (SE) and model fit statistics; ARIC (1987-89)

	Factor Loadings (SE)								Model fit statistics			
	$\alpha_{1Q} = \lambda_i$		α_{1M}		α_{1N}		α_{1T^*}		χ^2 (d.f.)		RMSEA	(90% CI)
6P	0.241	(0.019)	0.996	(0.026)	0.864	(0.024)	-0.067	(0.019)	5.9	(5)	0.008	(0.000, 0.028)
3P	0.174	(0.033)	0.482	(0.077)	0.520	(0.096)	0.415	(0.067)	0.0	(4)	0.000	(0.000, 0.000)
6H	0.079	(0.019)	1	…	0.898	(0.008)	0.017	(0.019)	18.6	(8)	0.022	(0.009, 0.035)
3H	0.389	(0.017)	0.870	(0.009)	1	…	0.058	(0.019)	7.2	(8)	0.000	(0.000, 0.020)
6	0.268	(0.020)	0.875	(0.029)	0.823	(0.029)	-0.039	(0.021)	4.7	(5)	0.000	(0.000, 0.025)
3	0.313	(0.019)	0.900	(0.021)	0.942	(0.021)	0.110	(0.021)	8.1	(5)	0.015	(0.000, 0.033)
3+6	0.223	(0.018)	1	…	0.758	(0.012)	-0.060	(0.019)	3.9	(8)	0.000	(0.000, 0.012)
3P/6P	0.363	(0.026)	0.748	(0.042)	0.595	(0.045)	0.472	(0.030)	0.0	(4)	0.000	(0.000, 0.000)
3H/6H	0.443	(0.033)	0.779	(0.051)	0.938	(0.059)	0.322	(0.027)	0.0	(4)	0.000	(0.000, 0.000)
3/6	0.364	(0.019)	0.956	(0.020)	0.876	(0.020)	0.569	(0.019)	5.8	(5)	0.007	(0.000, 0.028)

* Models with d.f=4 estimated Corr(ε_M, ε_N), Corr(ε_N, ε_{T^*}) and had no constraints on error variances or factor loadings. Models with d.f.=5 had a constraint on Corr(ε_M, ε_N)=0. Models with d.f.=8 had constraints on both Corr(ε_M, ε_N)= Corr(ε_N, ε_{T^*})=0 and error variance of either N or M ≈0 and α_{1M} or α_{1N} = 1. †A sensitivity analysis in models with correlated errors constraints showed that constraining correlations to non-zero values had little effect on the estimate of λ_i. Cov(ε_M, ε_N) was varied between -0.1 and 0.1 in increments of 0.05 for models with 5 degrees of freedom. Results of this sensitivity analysis can be provided upon request.

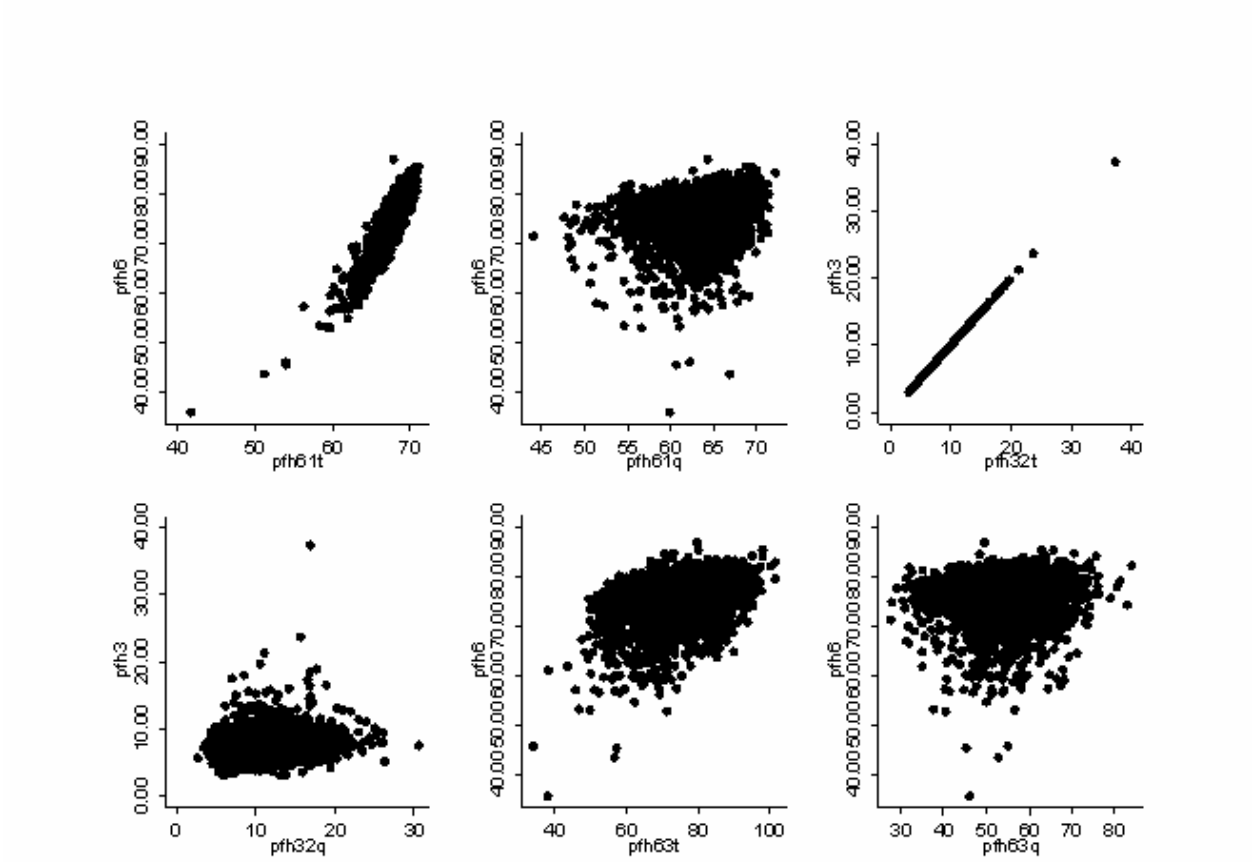
TABLE 5.1.4.

Variance-covariance and correlation matrix of measurement error ($\hat{\Sigma}_{uu}$) in measured variables Q_j; ARIC (1987-89)

	$\hat{\Sigma}_{uu}$									
Variable *j*	6P	3P	6H	3H	6	3	3+6	3P/6P	3H/6H	3/6
6P	(1.506) †	*0.164**	*-0.214*	*-0.090*	*0.914*	*-0.026*	*0.940*	*-0.360*	*0.038*	*-0.452*
3P	0.207‡	(1.054)	*0.015*	*0.006*	*0.111*	*0.456*	*0.191*	*0.527*	*-0.034*	*0.223*
6H	-0.356	0.021	(1.842)	*0.347*	*0.025*	*0.330*	*0.028*	*-0.107*	*-0.094*	*0.188*
3H	-0.122	0.007	0.521	(1.222)	*-0.0762*	*0.864*	*0.048*	*0.123*	*0.816*	*0.689*
6	1.286	0.131	0.038	-0.097	(1.315)	*-0.019*	*0.958*	*-0.440*	*-0.050*	*0.475*
3	-0.037	0.535	0.511	1.091	-0.025	(1.305)	*0.129*	*0.341*	*0.671*	*0.742*
3+6	1.439	0.245	0.047	0.129	-0.480	0.370	(1.555)	*-0.360*	*0.105*	*0.682*
3P/6P	-0.421	0.514	-0.138	0.066	1.369	0.184	-0.427	(0.906)	*0.105*	*0.682*
3H/6H	0.047	-0.034	-0.129	0.911	-0.056	0.775	0.093	0.101	(1.021)	*0.548*
3/6	-0.609	0.251	0.280	0.837	-0.599	0.932	-0.463	0.713	0.608	(1.207)

* Numbers in italics constitute the correlation coefficients of measurement error between variables *j*. † Numbers in parentheses constitute the variance of error for variable *j*.
‡ Numbers that are non-italicized are the covariances between measurement errors of variables *j*.

FIGURE 5.1.1. Scatter plot of predicted (x-axis) vs. observed (y-axis) *pfh3* and *pfh6* biomarkers: Improvement in model fit between "T*" and "Q" combinations; ARIC (1987-89)*



* pfh_6: Observed value of the pfh6 biomarker; pfh_3: Observed value of the pfh3 biomarker; pfh_{61t}: predicted value of pfh_6 based on Eq. 5.2.1 using the combination of alloyed gold standard intake values T^*; ; pfh_{61q}: predicted value of pfh_6 based on the empirical equation 5.2.1 using the combination of error-prone intake values Q; The same principle applies to pfh_{32t} vs. pfh_{32q} and pfh_{63t} vs. pfh_{63q} although these are predicted values from empirical equations 5.2.2 and 5.2.3.

B. ω-3 fatty acids, hypertension and risk of cognitive decline among older adults in the Atherosclerosis Risk in Communities (ARIC) study

Recent research indicates that ω-3 fatty acids can inhibit cognitive decline, perhaps differentially by hypertensive status. We tested these hypotheses on men and women of the ARIC cohort aged 50 years or older with complete cognitive status and dietary data (n=7,814) and a subset with plasma fatty acid data (n=2,251). Dietary assessment using a food frequency questionnaire and plasma exposure by gas chromatography were completed in 1987-89, while cognitive assessment with three screening tools, namely Delayed Word Recall Test (DWRT), Digit Symbol Substitution Test/Wechsler Adult Intelligence Test-Revised (DSST/WAIS-R) and the word fluency test (WFT), was completed both in 1990-92 and 1996-98. Results indicate that higher dietary intake of long chain ω-3 fatty acids as well as balancing long-chain ω-3/ω-6 decreased the risk of six-year cognitive decline in verbal fluency, particularly among hypertensive subjects. This finding was confirmed in the corresponding exposures in the plasma cholesteryl ester and phospholipid fractions. A sensitivity analysis using regression calibration and simulation extrapolation showed that there was a substantial amount of attenuation in effect of the dietary exposures on cognitive decline as well as loss of statistical power to detect significant associations.

B.1. Introduction

The proportion of the US population ages 65 and over was 12.3 percent in 2000 based on recent United Nations estimates and is projected to increase rapidly in the coming decades to reach 20 percent by 2050 (1). As populations age, all cognitive disorders, including dementia, become more common. Even more commonly, older persons can develop demonstrable cognitive impairment, especially memory deficits, without crossing the threshold of dementia. This condition has been termed "mild cognitive impairment" (MCI)(88).

Hence, MCI often represents a transitional state between the cognitive changes of normal aging and very early dementia and has become recognized as a risk factor for Alzheimer disease (AD). Identifying cognitive decline at a preclinical phase such as MCI would likely increase the efficacy of treatment.

Several neuropsychological tests and biomarkers have been developed to predict probability of progression from MCI to AD and other dementias (178). These tests can also be used in epidemiological studies to assess cognitive decline with respect to potential risk factors, health behaviors, and preventive treatments among middle aged and older adults.

Recent research indicates that ω-3 fatty acids may be important in preventing cognitive decline. So far, epidemiological evidence, although inconclusive, suggests a protective effect of ω-3 fatty acid intake in the diet (137, 388, 389). Despite animal experimental evidence of biological interaction between dietary intake of ω-3 fatty acids and hypertensive status (29), no epidemiological study to date has attempted to test this hypothesis. The present observational prospective study assessed the effect of low ω-3 fatty acid status on six-year cognitive decline in men and women aged 50 years and older, as well as the interaction of this risk factor with elevated blood pressure.

B.2. Materials and Methods

B.2.1. Study Sample

Atherosclerosis Risk in Communities (ARIC) is an ongoing prospective cohort study aimed at investigating the etiology of atherosclerosis and its clinical sequelae and the longitudinal impact of variation in cardiovascular risk factors, medical care, and disease by race, sex, place, and time.

In each of four US communities--Forsyth County (NC), Jackson (MS), suburbs of Minneapolis (MN), and Washington County (MD)-- 4,000 adults aged 45-64 years were examined four times, three years apart (visits 1 through 4). Three out of the four cohorts represented the ethnic mix of their communities, while at Jackson, MS, only African American residents were recruited (403). Out of the total sample examined at baseline (N=15,792) we restricted these analyses to 11,557 individuals aged 50 years or older at baseline since research clearly shows that risk of cognitive decline in general, and of dementia in particular, is negligible prior to the age of 60 years (which is the age at which the youngest individuals in this cohort were re-examined in visit4) (404). Further, it was shown in this cohort (44) that the effect of hypertension on cognitive decline is more pronounced among those aged 53 years or older at visit 1. Eligibility for these analyses further required complete data on cognitive functioning at visits 2 (1990-92) and 4 (1996-98) and also complete dietary intake at visit 1 (1987-89), which yielded n=7,814 men and women. Of these, plasma fatty acid data at visit 1 was available on a sub-set of the Minneapolis cohort, MN (n=2,251).

B.2.2. Outcome Assessment

Three measures of cognitive functioning were made for visits 2 and 4 of the ARIC study, and these measures relied on the following instruments: Delayed Word Recall Test (DWRT) (167); the Digit Symbol Substitution portion of the Revised Weschler Adult Intelligence Scale (DSST/WAIS-R) (405), and Word Fluency Test (WFT) of the Multilingual Aphasia Examination, also know as the controlled oral word association (170).

The *Delayed Word Recall Test (DWRT)*: This screening tool assesses verbal learning and recent memory. It requires the respondent to recall 10 common words after a 5-minute interval during which another test is administered. Test scores may range between 0 and 10 words recalled and the time limit for recall is set at 60 seconds.

The 6-months test-retest reliability of DWRT was previously shown to be high among 26 normal elderly individuals (Pearson correlation coefficient, r=0.75) (167).

The *Digit Symbol Substitution (DSST/WAIS-R):* This test is a paper-and-pencil test requiring timed translation of numbers 1 through 9 to symbols using a key. The test measures psychomotor performance and is relatively unaffected by intellectual ability, memory, or learning for most adults(170). It appears to be a sensitive and reliable marker of brain damage(407). The test score can range between 0 and 93 and it refelects the correctly translated number of digit-symbol pairs within a time limit of 90 seconds. Short-term test-retest reliability over 2-5 weeks has been found to be high in individuals aged 45-54 years (r=0.82); (405).

The *Word Fluency Test (WFT):* This test requires subjects to record as many words as possible using the initial letters F, A and S and to list these words, the subject is given only 60 seconds per letter. The total score corresponds to the total number of words generated during these three trials. The test is particularly sensitive to linguistic impairment (170, 408) and early mental decline in older persons (409). It is also a sensitive marker of damage in the left lateral frontal lobe (170, 408). The immediate test-retest correlation coefficient based on an alternate test form has been found to be high (r=0.82); (410).

Cutoff points were determined for decline in each of three cognitive status tests using the Reliable Change Index (RCI) method in order to correct for measurement error and practice effects (411). RCI is defined as $((X_2-X_1)-(M_2-M_1))/S.E.D.$, where X_1 is the individual's score at baseline, X_2 the individual's score at follow-up, M_1 and M_2 are the group mean pretest and follow-up scores respectively, and S.E.D. the observed standard error of the difference scores. Scoring below an RCI of -1.645 was regarded as a "statistically reliable" deterioration in the test scores.

A composite measure of the three RCIs to assess global cognitive decline (GCD) was created using principal components analysis (PCA), a data reduction technique. Similarly, the cutoff point of the composite score for statistically reliable global cognitive decline was chosen to be -1.645.

B.2.3. Exposure Assessment

Usual dietary intake was estimated from an interviewer-administered 61-item semi-quantitative food frequency questionnaire (FFQ) previously developed and validated by W. Willet and colleagues against multiple food records among a sub-sample of the Nurse's Health Study cohort (416). This instrument was modified by adding five regional/ethnic food items. Interviewees were asked how often, on average, they had consumed certain foods in portions of a specified size during the preceding year. There were nine possible responses, ranging from "almost never" to "more than six times per day." Daily intake of nutrients was calculated by multiplying the nutrient content of each food in the portion specified by the frequency of daily consumption and summing the results. The nutrient content of each food was obtained from the Harvard nutrient data base for which the primary source was the Department of Agriculture handbook(417).

Dietary intake of essential fatty acids and their elongated and desaturated products were expressed as percent of total energy intake and grouped under four main categories, as suggested by Lands and colleagues (381, 382): **(3P)** ω-3 C_{18} polyunsaturated fatty acids: 18:3+18:4ω-3 **(6P)** ω-6 C_{18} polyunsaturated fatty acids: 18:2+18:3ω-6 **(3H)** ω-3 C_{20} and C_{22} highly unsaturated fatty acids (HUFAs): 20:5+22:5+22:6ω-3 and **(6H)** ω-6 HUFAs: 20:3+20:4+22:4+22:5ω-6. Sums of fatty acid intake as percent of energy included (3)= (3P)+(3H) and (6)=(6P)+(6H). Ratios of interest included (3P)/(6P), (3H)/(6H) and (3P+3H)/(6P+6H) also denoted as 3/6. In multivariate models, all these variables were standardized by subtracting each observation from the variable mean and dividing the difference by the standard deviation. Hence, the main exposures of interest were 3P, 3H, 3 (as percent of energy intake), 3P/6P, 3H/6H, 3/6, and total 3H (in grams per day). Adjustment was made for the other fatty acid variables when appropriate, and total energy intake was considered as a potential confounder to emulate a multivariate nutrient density model(457).

Twelve-hour fasting blood was collected according to the ARIC study wide protocol. The Minneapolis field center conducted fatty acid analysis of plasma phospholipid and cholesteryl ester fractions on visit 1 blood specimens. The procedure is described in detail elsewhere (422). The identity of 28 fatty acid peaks were revealed by gas chromatography by comparing each peak's retention time to the retention times of fatty acids in synthetic standards of known compositions. The relative amount of each fatty acid (as a percent of all fatty acids) was calculated by integrating the area under the peak, dividing the result by the total area for all fatty acids, and multiplying by 100. Data from the chromatogram were transferred electronically to a computer for analysis.

Plasma exposures are expressed as % of total fatty acids in each fraction and were grouped similarly to dietary exposure. However, only 3H and the ratio of 3H/6H were considered in these analyses.

B.2.4. Covariates

Most covariates considered were measured at visits 1 or 2, although some were defined according to criteria that spanned all four visits. Covariates can be subdivided into socio-demographic, genetic, health behaviors, nutritional, and co-morbid conditions or medications. Age, gender, ethnicity and education were all reported by the respondent.

Apo E genotype was categorized as 0 to indicate the absence of an ε4 allele vs.1 to denote carrier status for at least one ε4 allele. Among the behavioral factors (all measured at visit 1), smoking was represented on a three-level categorical scale, namely: never smoked, smoked previously and current smoker. Food frequency questionnaire derived values of alcohol (grams/day) and caffeine (mg/day) were considered as well. Physical activity was assessed using a questionnaire developed by Baecke and colleagues, including 16 items about usual exertion (424). A validated index of physical activity was derived at visit 1, summing sports, work and leisure indices which ranged from a score of 1 (low) to 5 (high) (425). Body mass index at visit 1 was computed by dividing weight in kilograms by the height-squared (in square meters). Baseline dietary intake of antioxidants and other micronutrients (mainly Vitamins B_6 , B_{12} and folate) was considered as well (416).

A number of co-morbid conditions were also measured for descriptive purposes namely: Stroke or TIAs, type II diabetes mellitus (defined as fasting blood glucose ≥ 140 mg./dl or self-reported diabetes or use glucose lowering medication) and dyslipidemia (fasting blood HDL-C<40 (men) or <50 (women) and triacylglycerols>150 mg/dl as recommended by NCEP (426)) at any visit, hypercoagulable profile (upper quintile of at least two of: fibrinogen, vWF and factor VIII), poor pulmonary function (ratio $FEV_1/FVC \leq 0.70$ as measured by a spirometer at visits 1 or 2), and depressive symptoms at visit 2 using a 21-item vital exhaustion scale (427, 428). Medications considered included statins or medications known to lower cholesterol, NSAIDs and psychotropic drugs, screened at all visits. The association of these covariates with our outcome has been previously documented by similar cohort studies based on ARIC data (150, 197, 216, 406). In addition, baseline cognitive score was controlled for when needed. For GCD, a composite baseline score of RCIs using PCA was added to the model.

Our main effect modifier, hypertension, was operationalized using measured systolic and diastolic blood pressure at each visit as well as use of anti-hypertensive medication over the past two weeks. Seated blood pressure levels were calculated as the average of the second and third of three consecutive measurements with a random-zero sphygmomanometer and hypertension was defined as ≥ 140 mm Hg. for SBP and ≥ 90 mm Hg. for DBP or the use of anti-hypertensive medication during the past two weeks prior to examination on any of visits 1 through 4.

B.2.5. Statistical Analysis

We carried out univariate analyses of predictor and outcome variables as well as covariates. For bivariate analyses of exposure and outcome, we computed means of predictor variables across outcome groups (0 = no decline; 1= decline) and assessed statistical significance of differences using independent samples *t*-test at an alpha level of 0.05.

We computed odds ratios of decline with increase in each exposure by 1 SD through a multivariate logistic regression analysis. Control for confounding was accomplished using backward elimination and an overall change in estimate criterion of 5%. Covariates which changed the estimated effect of the exposure by more than 5% were retained in the final model(458).

Covariates considered as potential confounders were: baseline cognitive functioning, socio-demographics (age, sex, education, race), genetic factors (Apolipoprotein E ε4 allele); behavioral factors (smoking, alcohol and caffeine consumption and physical activity) and nutritional factors (body mass index, caloric intake, other fatty acids, intake of antioxidants and vitamins B_6, B_{12} and folate, other dietary fatty acids).

Hypertension was considered as a potential effect modifier. Likelihood ratio tests were used to assess statistical significance of interaction between exposure and hypertensive status at a type I error level of 0.20, after obtaining the final parsimonious model.

All other covariates were deemed as potential intermediates and hence were not included in the multivariate models. The multivariate models can be summarized by equations **(5.9.1)** and **(5.9.2)**:

(5.9.1) $$\text{Logit}[\Pr(Y=1 \mid Q, Z)] = \beta_0 + \beta_1 Q_1 + \Sigma\beta_{2i} Q_{2i} + \Sigma\beta_j Z_j$$

(5.9.2) $$\text{Logit}[\Pr(Y=1 \mid Q, Z, H)] = \beta_0 + \beta_1 Q_1 + \Sigma\beta_{2i} Q_{2i} + \beta_3 H + \Sigma\beta_j Z_j + \gamma Q_1 \times H$$

In the above equations, Logit is the $\text{Log}_e[\Pr/(1-\Pr)]$, Y is the binary outcome of "statistically reliable" cognitive decline (RCI<-1.645), Q_1 is the main exposure of interest as derived from the food frequency questionnaire, Q_{2i} are the other fatty acids that might act as confounders, Z_j is a vector of potential confounders that are assumed to be perfectly measured, and H is the effect modifier "hypertensive status" also assumed to be perfectly measured. The same process was used with the plasma exposures in cholesteryl esters and phospholipids.

To correct for measurement error in dietary exposure, a sensitivity analysis was conducted for models **(5.9.1)** and **(5.9.2)** whereby regression calibration and simulation extrapolation were applied to the final parsimonious models for each outcome/exposure pair (449, 450). The two methods are described in more detail in Appendix A. Statistical analyses were conducted using STATA ver. 8.2 (412).

B.3. Results

Table 5.2.1 shows the characteristics of study subjects according to availability of dietary and plasma fatty acid data as well as cognitive assessment data at both points in time. Subjects in the plasma fatty acid group consisted of whites residing in the suburbs of Minneapolis. They were in general more educated than the dietary group, which was a mix from all ARIC centers. They had a lower proportion of women (50.7% vs. 54.63%), a lower prevalence of the ApoEε4 allele (28.8% vs. 30.0%), a higher proportion "ever smoked" status (59.5% vs. 55.4%), and greater consumption of alcohol and caffeine. Some differences were noted for other behavioral and nutritional factors as well. Most co-morbid conditions were more prevalent in the dietary group, with the exception of dyslipidemia and poor pulmonary function which were similar for both groups. Use of psychotropic drugs, NSAIDs and statins over the span of follow-up were relatively high in both groups. Hypertensive status was particularly high in the dietary group (56%) compared to 49% among the plasma group. Raw mean scores of baseline cognitive function were greater among those in the plasma compared to the dietary group and average declines between visits 2 and 4 were found to be steeper in the former.

Unadjusted mean levels of ω-3 fatty acids by cognitive decline and hypertensive status are presented in Table 5.2.2. Daily intake of long chain ω-3 fatty acids (*3H* in milligrams) was significantly lower among subjects whose scores declined on the word fluency test when compared to those who didn't. However, this protective effect was seen only among hypertensive individuals. In addition, the ratio of long chain ω-3 to long chain ω-6 was also protective against decline both among hypertensive subjects and the total study population, but not among normotensives. It is worth noting that in general, hypertensive subjects tended to have a significantly higher intake of long chain and that total ω-3 fatty acids and their ratio of ω-3 / ω-6 fatty acids were consistently higher when compared to normotensives. In terms of plasma exposures, the same pattern was observed, in that there was no significant effect in normotensives but a clear protective effect among the total study population and the hypertensive subgroup. For both types of exposures (diet and plasma), the effect on other areas of cognition as well as the global cognitive decline outcome did not reach statistical significance.

Multivariate logistic regression of the relationship between cognitive decline and dietary exposure is presented in Table 5.2.3. Results indicate that risk of clinically significant decline in DWRT over the period of 6 years was reduced modestly with every standard deviation increase in long-chain ω-3 fatty acid intake (*3H*) as % of total energy intake. This was observed in the total population and among the hypertensive subgroup. For DSST/WAIS-R, although the ratio *3H*/*6H* was protective against decline, this effect did not reach statistical significance. However, the likelihood ratio test indicated a significant level of interaction between this exposure and hypertensive status, shown by the variation in effect across strata of the effect modifier (1.09 among normotensives *vs.* 0.88 among hypertensives).

Risk of decline in WFT was reduced by long chain and all types of ω-3 fatty acid intake (*3H* and *3*) as % of total energy intake, and by the ratio of 3H/6H and 3H in milligrams. This relationship was stronger among hypertensive subjects and a significant interaction was noted for *3H* in milligrams. No statistically significant results were observed for global cognitive decline or other dietary exposures. After adjusting for measurement error in the dietary covariate using regression calibration, loss of precision in measures of effect was observed. In most cases, bias seemed to be towards the null when comparing naïve and calibrated estimates. Using SIMEX, similar results were obtained, although bias was shown to be more severe while precision was less affected. Figure 5.2.1 shows two examples of stratified models 3.2b and 3.2g of Table 5.2.3 (3H and ratio of 3H/6H's effect on decline in WFT). The figures show the extent to which naïve estimates are biased towards the null when compared to the corrected regression coefficients using the SIMEX method. This method is described in detail in Appendix A.

Multivariate logistic analyses of the plasma fatty acid data (Table 5.2.4) indicated generally lower odds of cognitive decline among subjects with a higher concentration of long chain ω-3 fatty acid in their plasma cholesteryl esters and phospholipids, and an elevated ratio of long chain ω-3/ ω-6 fatty acids. This was confined to word fluency however, although an interaction with hypertensive status was found for DSST/WAIS-R for cholesteryl ester 3H/6H ratio. This interaction was also strong and in the same direction for WFT for absolute 3H in cholesteryl esters (OR: 0.63 among normotensives vs. 0.35 among hypertensives). The same pattern was observed for the ratio of 3H/6H in cholesteryl esters. For phospholipids, interaction with hypertensive status did not reach statistical significance (LR test p-value > 0.20). As mentioned, global cognitive decline was not affected by any of the exposures, although an interaction was found for 3H in cholesteryl esters.

B.4. Discussion

This population-based prospective study conducted among middle-aged men and women at baseline showed that increased dietary intake of long chain ω-3 fatty acids and balancing long-chain ω-3/ω-6 decreased the risk of cognitive decline in verbal fluency, particularly among hypertensive subjects. This finding also held for the corresponding plasma analytes in the cholesteryl ester and phospholipid fractions. Limitations of the study include the lack of psychometric diagnosis for mild cognitive impairment, which might have been a more definite and clinically relevant outcome. However, the neuropsychological tests used represent some of the domains reported to be most sensitive in discriminating between normal aging and mild cognitive impairment (110). One of the main strengths of this study is its prospective design which, as stated earlier, thus far is unique in the literature testing this particular hypothesis (459). In addition, an evidence-based report suggested a need to look for the effect of ω-3 fatty acids on cognitive decline by cardiovascular disease status and to define exposure in terms of absolute value of medium chain and long chain fatty acids, as well as the ratio between ω-3 and ω-6 fatty acids in diet and plasma (460). All these suggestions were implemented in the present study. Moreover, this is the first study to assess effect modification by hypertensive status and to test at the population level a biological interaction documented in animal experimental work. Measurement error, which almost always accompanies dietary assessment, was corrected for in this study using regression calibration and SIMEX.

Previous epidemiological studies have shown that the fatty acids composition of plasma differs significantly between subjects with normal cognitive functioning and patients with some form of cognitive impairment (384-386).

While the majority showed a beneficial effect of plasma and erythrocyte ω -3 fatty acids on cognition, a case-control study – the Canadian Study of Health and Ageing – reported that the mean relative plasma concentration of ω-3 fatty acids as well as total polyunsaturated fatty acids was higher among subjects aged 65 years or more with cognitive impairment or dementia after controlling for demographic, behavioral and genetic factors (387). Epidemiological studies based on dietary assessments of ω-3 fatty acids also had suggestive but somewhat controversial results. While most leaned towards a protective effect of increasing intake of these fatty acids in the diet (388-390, 461, 462), others found no effect or the opposite effect on cognitive functioning and decline (137, 141).

Essential fatty acids (Linoleic and α-linolenic acid) and their desaturated and elongated products (long chain ω-6 and ω-3 fatty acids) are linked to several biochemical and biophysical functions, including structural integrity and fluidity of membranes, enzyme activities, lipid-protein interactions and serving as precursors for eicosanoids such as prostaglandins, leukotrienes and thromboxanes (10). The fatty acid composition of neuronal cell membrane phospholipids reflects their intake in the diet (19) and fish oils, which contain high levels of C_{20} and C_{22} polyunsaturated fatty acids (PUFAs), exert the most profound influence on brain PUFA concentration. According to experimental animal studies, there is a plausible pathway by which hypertension and low dietary ω-3 fatty acid intake may interact in increasing the risk of cognitive decline. In fact, hypertensive rats tended to have lower brain MUFAs and PUFAs than normotensive rats (29), possibly due to pressure-induced endothelial dysfunction at the blood-brain barrier or exhausted astrocytic metabolism. Oxidative stress which accompanies high blood pressure leads to increased peroxidation of unsaturated fatty acids and a reduction in their concentration in the brain represents an alternative explanation.

One possibly important implication from our study's results is that diets rich in fatty acids of marine origin should be considered for middle aged hypertensive subjects. The observed associations among normotensive subjects was also suggested protection as regards cognitive decline, albeit to a lesser extent and without reaching nominal levels of statistical significance. These results merit replication given the large public health potential that would be associated with results that unequivocally indicate inverse association between fatty acid intake and reduced cognitive decline in the general population. The literature indicates that these fatty acids were frequently found associated with reduced risk of cardiovascular disease, including stroke (327) and coronary heart disease (324, 325), although thus far all the evidence is of an observational nature. They have also been associated with improved insulin sensitivity(341), reduced risk of dyslipidemia (346) and a hypocoagulable profile (332) among other health benefits. Because many of these conditions are also related to cognitive impairment, future research should focus on disentangling the direct and indirect effects of fatty acids (using plasma biomarkers) on cognition and uncover the main mechanism involved in their ability to prevent clinically significant decline in aging populations. Finally, these findings suggest the utility of randomized clinical trials that would augment intake of marine fatty acids in the treatment group and give a non-enriched diet to the placebo group while allowing for stratification by baseline hypertensive status.

TABLE 5.2.1.

Characteristics of study subjects with complete cognitive and dietary data between visits 1 and 4 (Dietary group; N=7,814) and those with complete cognitive and plasma data (Plasma group; N=2,251); ARIC 1987-98

Characteristics	Dietary group (All ARIC centers) Mean (%)	(SD)	Plasma group (MN whites) Mean (%)	(SD)
Female	54.63		50.69*	
Age (years)†	56.56	(4.31)	56.30*	(4.24)
White†	81.48		100.00*	
Education†				
Incomplete high school	20.24		6.67*	
High School	34.06		36.18	
> High School	45.70		57.16	
Apo E ε4 allele†	30.00		28.84	
Smoking status†				
Never smoker	44.54		40.40*	
Former smoker	35.55		41.82	
Current smoker	19.91		17.78	
Alcohol (g/day)†	5.88	(12.67)	8.08*	(13.47)
Caffeine (mg/day)†	291.04	(290.82)	348.08*	(325.93)
Physical activity scale†	7.06	(1.39)	7.33*	(1.33)
Body mass index (kg/m^2)†	27.48	(4.96)	27.17*	(4.41)
Total energy intake (Kcal/day)†	1579	(571)	1581	(559)
Vitamin A (in 1000 IUs/day)†	9.13	(6.97)	8.65*	(6.83)
Vitamin B_6 (mg/day)†	1.75	(0.67)	1.74*	(0.66)
Vitamin B_{12} (mcg/day)†	7.61	(4.23)	7.06*	(3.50)
Vitamin C (mg/day)†	122.39	(80.92)	112.67*	(69.95)
Vitamin E (mg/day)†	4.97	(3.11)	4.66*	(3.01)
Folate (mcg/day)†	232.59	(101.18)	218.48*	(94.97)
Stroke/TIAs‡	9.92		9.28	
Depression scale§	9.82	(8.19)	8.19*	(7.12)
Type II Diabetes‡	18.25		13.86*	
Dyslipidemia‡	36.61		37.05*	
Poor pulmonary function (FEV_1/FVC <70)§	17.88		18.93	
Hypercoagulable profile†	15.33		11.11*	
Fibrinogen value	300.09	(61.80)	294.44*	(60.96)
vWF	117.38	(44.80)	111.14*	(39.67)
Factor VIII	129.83	(35.89)	124.28*	(32.70)
Use of psychotropic medication‡	78.77		77.61	
Use of NSAIDs‡	73.91		78.50*	
Use of statins‡	51.61		47.09*	
Hypertensive‡	56.01		49.27*	
Baseline cognitive scores (V2)§				
DWRT	6.61	(1.46)	6.78*	(1.43)
DSST/WAIS-R	45.02	(13.01)	51.65*	(10.15)
WFT	33.77	(12.06)	37.59*	(11.52)
Cognitive change (V4-V2)§				
DWRT	-0.17	(1.56)	-0.21	(1.50)
DSST/WAIS-R	-2.80	(6.79)	-4.45*	(6.36)
WFT	-0.77	(7.86)	-1.84*	(7.78)

*p<0.05 for null hypothesis that means or proportions are equal between plasma and non-plasma groups; † Covariate measured at visit 1; ‡Covariate measured at visits 1 through 4: period prevalence over 9 years; § Covariate with other time frame.

TABLE 5.2.2.
Unadjusted mean (SD) of ω-3 fatty acid exposures by cognitive decline status in three domains (DWRT, DSST/WAIS-R, WFT) and global cognitive decline (GCD), stratified by hypertensive status; ARIC 1987-98

			Statistically reliable cognitive decline (RCI<-1.64)							
			DWRT		DSST/WAIS-R		WFT		GCD	
	Mean	(SD)	No	Yes	No	Yes	No	Yes	No	Yes
Dietary Intake (% of energy)										
All Subjects										
3P	0.415	(0.086)	0.415	0.416	0.415	0.412	0.415	0.416	0.414	0.413
3H	0.182	(0.166)	0.182	0.174	0.182	0.181	0.182	0.170	0.182	0.178
3	0.597	(0.190)	0.597	0.590	0.597	0.593	0.597	0.586	0.597	0.592
3P/6P	0.103	(0.037)	0.103	0.103	0.103	0.104	0.103	0.103	0.103	0.103
3H/6H	2.272	(1.872)	2.277	2.199	2.269	2.320	2.284	2.052*	2.276	2.216
3/6	0.147	(0.073)	0.147	0.144	0.147	0.148	0.147	0.143	0.147	0.145
3H (in mg/day)	302	(283)	302	300	302	294	303	279	302	292
Normotensive										
3P	0.415	(0.089)	0.415	0.409	0.415	0.404	0.415	0.420	0.415	0.413
3H	0.173	(0.162)	0.173	0.163	0.172	0.180	0.173	0.164	0.173	0.163
3	0.588	(0.187)	0.588	0.573	0.588	0.585	0.588	0.585	0.588	0.576
3P/6P	0.101	(0.037)	0.101	0.099	0.101	0.101	0.101	0.102	0.101	0.099
3H/6H	2.215	(1.775)	2.215	2.210	2.209	2.360	2.223	2.059	2.223	2.054
3/6	0.142	(0.069)	0.142	0.137	0.142	0.142	0.142	0.141	0.143	0.135
3H (in mg/day)	286	(265)	285	299	286	299	286	288	286	289
Hypertensive†										
3P	0.415	(0.085)	0.414	0.422	0.415	0.416	0.415	0.413	0.415	0.415
3H	0.189*	(0.169)	0.190	0.178	0.190	0.182	0.190	0.172	0.189	0.189
3	0.604*	(0.193)	0.604	0.600	0.605	0.599	0.605	0.584	0.604	0.603
3P/6P	0.104*	(0.037)	0.104	0.106	0.104	0.106	0.104	0.102	0.104	0.105
3H/6H	2.310*	(1.817)	2.328	2.188	2.331	2.130	2.334	2.025*	2.328	2.194
3/6	0.150*	(0.076)	0.151	0.149	0.150	0.150	0.151	0.142	0.150	0.151
3H (in mg/day)	315*	(298)	316	300	316	292	318	265*	317	300
Plasma Cholesteryl Esters‡										
All Subjects										
3H	1.009	(0.395)	1.008	1.015	1.009	1.010	1.012	0.947	1.006	1.044
3H/6H	0.112	(0.047)	0.112	0.109	0.112	0.112	0.113	0.101*	1.112	1.111
Normotensive										
3H	0.997	(0.408)	0.995	1.029	0.995	1.026	0.997	0.976	0.992	1.072
3H/6H	0.113	(0.048)	0.113	0.114	0.113	0.117	0.113	0.106	0.113	0.118
Hypertensive										
3H	1.024	(0.387)	1.026	1.004	1.026	0.991	1.030	0.922*	1.024	1.023
3H/6H	0.112	(0.045)	0.113	0.106	0.113	0.107	0.113	0.097*	0.113	0.105
Plasma Phospholipids‡										
All Subjects										
3H	3.444	(1.053)	3.448	3.379	3.442	3.482	3.453	3.279	3.437	3.557
3H/6H	0.222	(0.082)	0.223	0.214	0.222	0.222	0.223	0.206*	0.222	0.223
Normotensive										
3H	3.438	(1.059)	3.437	3.460	3.434	3.513	3.440	3.381	3.426	3.634
3H/6H	0.225	(0.084)	0.225	0.225	0.225	0.231	0.226	0.217	0.225	0.226
Hypertensive										
3H	3.458	(1.053)	3.468	3.314	3.460	3.433	3.472	3.218	3.454	3.518
3H/6H	0.220	(0.080)	0.220	0.206	0.220	0.213	0.221	0.198*	0.220	0.213

*p<0.05 for null hypothesis that means of exposures are equal to eachothers between cognitive decline categories or hypertensive status.
† "hypertensive": screened positive on measured hypertension at either visits 1 through 4 or were taking anti-hypertensive medication over the past two weeks prior to examination at any of the four visits. ‡ Plasma cholesteryl ester levels of fatty acids (%);Plasma phospholipids levels of fatty acids (%).

TABLE 5.2.3.

Multivariate Logistic models of cognitive decline and dietary ω-3 fatty acid exposures†: Interaction with hypertensive status: naïve and regression calibrated odds ratios; ARIC (1987-98)

Statistically reliable cognitive decline (RCI<-1.64)

	DWRT				DSST/WAIS-R				WFT			
	Naïve		RCAL§		Naïve		RCAL		Naïve		RCAL	
Dietary fatty acids	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
	Models 1.1a-1.1g				Models 2.1a-2.1g				Models 3.1a-3.1g			
All Subjects												
3P	1.02	(0.93, 1.12)	1.01	(0.72, 1.43)	0.96	(0.87, 1.07)	0.94	(0.63, 1.40)	1.01	(0.92, 1.12)	1.01	(0.68, 1.48)
3H	0.90*	(0.81, 1.00)	0.92	(0.72, 1.18)	0.92	(0.81, 1.03)	0.93	(0.70, 1.24)	0.85*	(0.75, 0.96)	0.80	(0.58, 1.11)
3	0.96	(0.87, 1.06)	0.93	(0.72, 1.21)	0.90	(0.80, 1.02)	0.84	(0.58, 1.21)	0.87*	(0.77, 0.98)	0.76	(0.52, 1.13)
3P/6P	1.01	(0.92, 1.10)	1.00	(0.79, 1.25)	1.04	(0.94, 1.15)	1.08	(0.84, 1.38)	1.01	(0.91, 1.10)	0.99	(0.75, 1.28)
3H/6H	0.96	(0.87, 1.06)	0.91	(0.75, 1.12)	1.03	(0.93, 1.13)	1.06	(0.83, 1.35)	0.86*	(0.77, 0.97)	0.81	(0.63, 1.04)
3/6	0.92	(0.83, 1.02)	0.94	(0.80, 1.11)	1.01	(0.92, 1.12)	1.02	(0.87, 1.20)	0.95	(0.85, 1.06)	0.92	(0.74, 1.13)
3H (mg/day)	0.91	(0.81, 1.01)	—	—	0.97	(0.87, 1.07)	—	—	0.87*	(0.76, 0.99)	—	—
	Model 1.2a-1.2g				Model 2.2a-2.2g				Model 3.2a-3.2g			
Normotensive stratum												
3P	0.94	(0.80, 1.09)	0.90	(0.53, 1.54)	0.88	(0.74, 1.05)	0.96	(0.51, 1.81)	1.06	(0.92, 1.24)	1.04	(0.62, 1.74)
3H	0.91	(0.76, 1.07)	0.92	(0.62, 1.38)	1.00	(0.81, 1.20)	1.18	(0.75, 1.87)	0.88	(0.72, 1.07)	0.86	(0.50, 1.49)
3	0.91	(0.78, 1.08)	0.89	(0.57, 1.41)	0.94	(0.77, 1.15)	1.12	(0.63, 2.00)	0.93	(0.77, 1.12)	0.89	(0.51, 1.56)
3P/6P	0.94	(0.80, 1.10)	0.90	(0.61, 1.31)	0.98	(0.83, 1.16)	1.02	(0.69, 1.50)	1.04	(0.89, 1.21)	0.97	(0.66, 1.43)
3H/6H	1.00	(0.85, 1.17)	0.98	(0.72, 1.34)	1.09	(0.92, 1.28)	1.23	(0.90, 1.68)	0.90	(0.75, 1.08)	0.90	(0.61, 1.31)
3/6	0.88	(0.74, 1.05)	0.87	(0.66, 1.17)	1.00	(0.84, 1.19)	1.03	(0.76, 1.39)	0.98	(0.83, 1.16)	0.94	(0.66, 1.34)
3H (mg/day)	0.92	(0.77, 1.09)	—	—	1.05	(0.88, 1.25)	—	—	0.94	(0.78, 1.14)	—	—
Hypertensive stratum												
3P	1.09	(0.97, 1.23)	1.13	(0.71, 1.81)	1.00	(0.88, 1.15)	0.94	(0.55, 1.59)	0.97	(0.84, 1.12)	0.96	(0.54, 1.72)
3H	0.88*	(0.77, 1.00)	0.88	(0.64, 1.22)	0.88	(0.76, 1.02)	0.82	(0.56, 1.20)	0.79*	(0.66, 0.95)	0.71	(0.47, 1.07)
3	0.97	(0.86, 1.10)	0.94	(0.68, 1.30)	0.89	(0.77, 1.03)	0.73	(0.45, 1.17)	0.79*	(0.67, 0.94)	0.64	(0.36, 1.11)
3P/6P	1.07	(0.95, 1.20)	1.10	(0.82, 1.48)	1.05	(0.93, 1.19)	1.09	(0.77, 1.55)	0.95	(0.83, 1.10)	0.95	(0.64, 1.39)
3H/6H	0.92	(0.80, 1.05)	0.85	(0.63, 1.16)	0.88**	(0.76, 1.02)	0.80	(0.59, 1.10)	0.81*	(0.68, 0.96)	0.70	(0.48, 1.03)
3/6	0.95	(0.84, 1.07)	0.98	(0.81, 1.18)	1.00	(0.89, 1.13)	1.00	(0.81, 1.21)	0.97	(0.76, 1.03)	0.85	(0.66, 1.10)
3H (mg/day)	0.90	(0.78, 1.04)	—	—	0.92	(0.80, 1.05)	—	—	0.78**	(0.64, 0.94)	—	—

* $p<0.05$ for testing the null hypothesis that $\beta_1=0$. See equations (5.9.1) and (5.9.2); ** $p<0.20$ for testing the null hypotheses that $\gamma=0$ using the likelihood ratio test. See equation (5.9.2).

†Exposures were standardized by subtracting each observation from its mean and dividing it by its Standard Deviation.

‡ Control for confounding was done using backward elimination and an overall change in estimate criterion of 5%. Covariates which changed the estimate of exposure by more than 5% were retained in the final model. Covariates considered as potential confounders were: baseline cognitive functioning, socio-demographics (age, sex, education, race); genetic factors (ApoE ε4 allele); behavioral factors (smoking, alcohol, caffeine consumption and physical activity) and nutritional factors (body mass index, caloric intake, other fatty acids, intake of antioxidants and vitamins B_6, B_{12} and folate). Hypertension was considered as a potential effect modifier in models 1.2a through 4.2g. Multiplicative interaction was tested using the likelihood ratio test at an alpha level of 0.20.

§ RCAL: Regression calibrated odds ratio (adjusted for measurement error) with its 95% confidence interval. __ Regression calibration was not performed on 3H(mg/day) since we could only correct for error in dietary intake of essential fatty acids expressed as % of energy intake along with their biologically plausible ratios.

Table 5.2.3. *(Cont'd)*

Statistically reliable cognitive decline (RCI<-1.64)			
GCD			
Naïve		RCAL	
OR	95% CI	OR	95% CI
Models 4.1a-4.1g			
0.98	(0.90, 1.08)	0.92	(0.64, 1.33)
0.91	(0.82, 1.02)	0.93	(0.70, 1.24)
0.90	(0.81, 1.01)	0.84	(0.58, 1.22)
1.01	(0.92, 1.10)	0.98	(0.78, 1.27)
0.97	(0.88, 1.06)	1.00	(0.74, 1.31)
0.98	(0.89, 1.07)	1.00	(0.85, 1.18)
0.96	(0.87, 1.06)	—	—
Model 4.2a-4.2g			
0.98	(0.83, 1.14)	0.96	(0.53, 1.71)
0.92	(0.75, 1.12)	1.02	(0.62, 1.67)
0.91	(0.75, 1.11)	1.02	(0.56, 1.84)
0.94	(0.80, 1.11)	0.93	(0.62, 1.40)
0.90	(0.75, 1.07)	0.99	(0.68, 1.43)
0.88	(0.73, 1.06)	0.95	(0.68, 1.32)
1.01	(0.86, 1.20)	—	—
0.99	(0.88, 1.14)	0.90	(0.57, 1.43)
0.93	(0.81, 1.06)	0.90	(0.63, 1.28)
0.92	(0.80, 1.06)	0.79	(0.49, 1.29)
1.02	(0.91, 1.16)	1.00	(0.72, 1.37)
0.92	(0.81, 1.05)	0.85	(0.62, 1.17)
1.00	(0.90, 1.12)	1.00	(0.83, 1.21)
0.94	(0.84, 1.06)	—	—

* $p<0.05$ for testing the null hypothesis that $\beta_1=0$. See equations (5.9.1) and (5.9.2);

** $p<0.20$ for testing the null hypotheses that $\gamma=0$ using the likelihood ratio test. See equation (5.9.2).

†Exposures were standardized by subtracting each observation from its mean and dividing it by its Standard Deviation.

‡ Control for confounding was done using backward elimination and an overall change in estimate criterion of 5%. Covariates which changed the estimate of exposure by more than 5% were retained in the final model. Covariates considered as potential confounders were: baseline cognitive functioning, socio-demographics (age, sex, education, race); genetic factors (ApoE ε4 allele); behavioral factors (smoking, alcohol, caffeine consumption and physical activity) and nutritional factors (body mass index, caloric intake, other fatty acids, intake of antioxidants and vitamins B_6, B_{12} and folate). Hypertension was considered as a potential effect modifier in models 1.2a through 4.2g. Multiplicative interaction was tested using the likelihood ratio test at an alpha level of 0.20.

§ RCAL: Regression calibrated odds ratio (adjusted for measurement error) with its 95% confidence interval.

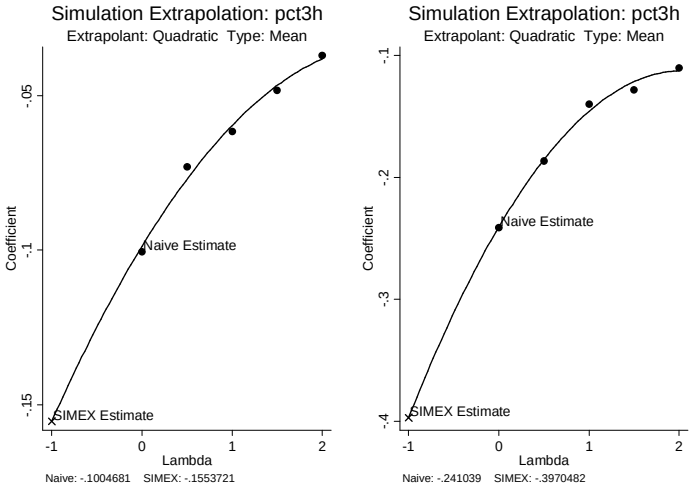
— Regression calibration was not performed on 3H(mg/day) since we could only correct for error in dietary intake of essential fatty acids expressed as % of energy intake along with their biologically plausible ratios.

TABLE 5.2.4.
Multivariate Logistic models of cognitive decline and plasma ω-3 fatty acid exposures: Interaction with hypertensive status: ARIC (1987-98)

	Statistically reliable cognitive decline (RCI<-1.64)							
	DWRT		DSST/WAIS-R		WFT		GCD	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Plasma cholesteryl ester§								
	Models 1.1a-1.1b		Models 2.1a-2.1b		Models 3.1a-3.1b		Models 4.1a-4.1b	
All Subjects								
3H	1.00	(0.82, 1.21)	0.91	(0.73, 1.14)	0.49*	(0.35, 0.70)	1.03	(0.87, 1.22)
3H/6H	0.92	(0.75, 1.14)	0.92	(0.74, 1.15)	0.51*	(0.36, 0.73)	0.97	(0.81, 1.16)
	Models 1.2a-1.2b		Models 2.2a-2.2b		Models 3.2a-3.2b		Models 4.2a-4.2b	
Normotensive stratum								
3H	1.09	(085, 1.40)	0.99	(0.74, 1.32)	0.64	(0.40, 1.01)	1.13	(0.92, 1.38)
3H/6H	1.02	(0.79, 1.32)	1.00	(0.76, 1.32)	0.65	(0.42, 1.03)	1.09	(0.88, 1.34)
Hypertensive stratum								
3H	0.89	(0.65, 1.21)	0.78	(0.55, 1.12)	0.37**	(0.22, 0.63)	0.92	(0.69, 1.22)
3H/6H	0.79	(0.56, 1.13)	0.76**	(0.51, 1.14)	0.38**	(0.22, 0.67)	0.78**	(0.55, 1.11)
Plasma phospholipids§								
	Models 1.3a-1.3b		Models 2.3a-2.3b		Models 3.3a-3.3b		Models 4.3a-4.3b	
All Subjects								
3H	0.93	(0.76, 1.14)	1.04	(0.87, 1.24)	0.67*	(0.52, 0.86)	0.96	(0.80, 1.15)
3H/6H	0.89	(0.73, 1.10)	0.99	(0.82, 1.20)	0.62*	(0.46, 0.83)	0.93	(0.77, 1.13)
	Models 1.4a-1.4b		Models 2.4a-2.4b		Models 3.4a-3.4b		Models 4.4a-4.4b	
Normotensive stratum								
3H	1.03	(0.77, 1.36)	1.07	(0.83, 1.39)	0.74	(0.52, 1.06)	1.03	(0.77, 1.36)
3H/6H	0.99	(0.75, 1.30)	1.06	(0.83, 1.37)	0.73	(0.50, 1.07)	1.04	(0.80, 1.35)
Hypertensive stratum								
3H	0.83	(0.61, 1.11)	0.97	(0.75, 1.26)	0.63*	(0.44, 0.92)	0.94	(0.73, 1.22)
3H/6H	0.77	(0.56, 1.08)	0.89	(0.66, 1.21)	0.56*	(0.35, 0.88)	0.86	(0.64, 1.15)

* $p<0.05$ for testing the null hypothesis that $\beta_1=0$. See equations (1) and (2); **$p<0.20$ for testing the null hypotheses that $\gamma=0$ using the likelihood ratio test. See equation (5.9.2). †Exposures were standardized by subtracting each observation from its mean and dividing it by its Standard Deviation. ‡ Same analytic approach was used as in Table 3. § Plasma cholesteryl ester levels of fatty acids (%);Plasma phospholipids levels of fatty acids.

Figure 5.2.1. SIMEX plot of corrected coefficients for model 3.2b and 3.2e of Table 3; ARIC (1987-98)*

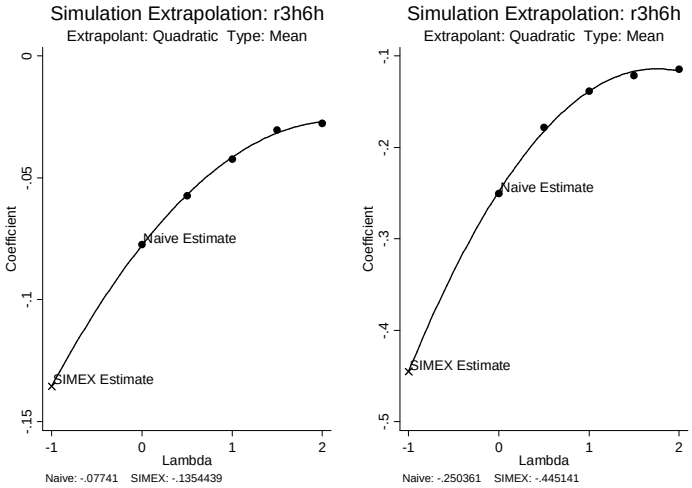


Normotensive stratum

SIMEX OR:0.86; 95% CI: (0.63, 1.15)

Hypertensive stratum

SIMEX OR: 0.67; 95% CI: (0.51, 0.89)



Normotensive stratum

SIMEX OR: 0.87; 95% CI: (0.64, 1.17)

Hypertensive stratum

SIMEX OR: 0.64; 95% CI: (0.47, 0.87)

*pct3h: dietary intake of long chain ω-3 fatty acids expressed as percentage of energy intake (3H); r3hr6h: ratio of long chain ω-3 to long chain ω-6 fatty acids (3H/6H). Lambda is equivalent to $\theta = \{.5, 1, 1.5, 2\}$ and is a scale factor used to add error to the covariate and estimate $\beta m = f(\theta, \beta m\{\theta\})$ starting from the naïve estimate in which $\theta = 0$. Hence, the naïve estimate of the regression coefficient β is the one estimated by generalized linear models without measurement error correction. See Appendix A for more details.

C. Plasma ω-3 fatty acids and risk of cognitive decline among older adults: an exploratory subgroup analysis

Plasma fatty acids may affect the risk of cognitive decline among middle-aged and older adults. We prospectively studied the association between plasma fatty acids and cognitive decline among older adults aged 50-65 years at baseline, and conducted an exploratory subgroup analysis. uring 1987-89, the ARIC study analyzed plasma fatty acids in cholesteryl esters and phospholipids among white subjects residing in Minneapolis. During 1990-92 and 1996-98, three neuropsychological tests in domains of delayed word recall (DWRT/WAIS-R), psychomotor speed (DSST/WAIS-R) and verbal fluency (WFT) were administered. We selected cutoff points for statistically reliable cognitive decline in each of these domains as well as a global measure of cognitive change (GCD) computed by principal components analysis. Multivariate logistic regression was conducted. Focusing on ω-3 highly unsaturated fatty acids (HUFAs), a subgroup analysis assessed differential association across potential effect modifiers implicated in oxidative stress and increased risk of neurodegenerative disease. Among the 2,251 study subjects, risk of global cognitive decline increased with elevated palmitic acid in both fractions, and high arachidonic acid and low linoleic acid in cholesteryl esters. Higher ω-3 HUFAs reduced risk of decline in verbal fluency, particularly among hypertensive and dyslipidemic subjects. No significant findings were shown for psychomotor speed or delayed word recall. Promoting higher intake of marine ω-3 fatty acids in the diet of hypertensive and dyslipidemic individuals might have substantial benefits in reducing their risk of cognitive decline in the area of verbal fluency. However, clinical trials are needed to confirm this finding.

C.1. Introduction

The effect of dietary intake on cognitive functioning has gained interest over the past few years. Several epidemiologic studies have shown that ω-3 fatty acids in blood differ significantly between subjects with normal cognitive functioning and patients with some form of cognitive impairment (384-386). These fatty acids in biomarkers of lipid intake have been historically associated with reduced risk of cardiovascular disease including stroke (327) and coronary heart disease (324, 325). They were also linked with improved insulin sensitivity (341), reduced risk of dyslipidemia (346), a hypocoagulable profile (332), improved pulmonary function (357) and reduced risk of major depression (352) among other health benefits.

Many of these conditions were related to increased oxidative stress (52, 396-400) which in turn causes neuronal loss and cognitive impairment among older adults (401). Consequently, it is essential to unveil any putative interaction between these conditions and the ability of this class of fatty acids to fulfill their beneficial effects. An additional genetic factor (ApoE ε4 allele) has been consistently associated with increased risk of cognitive decline (213, 216) and progression from pre-clinical stage to Alzheimer's disease (58). It has also been associated with increased level of oxidative stress (402) .

Our present study assesses the association of plasma cholesteryl ester and phospholipid levels of ω-3 fatty acids with decline in three areas of cognition. We conducted a subgroup analysis into the role of high levels of oxidative stress and risk of neurodegenerative disease. We hypothesized those subgroups with elevated physiological oxidative stress to benefit most from an increased intake of dietary ω-3 fatty acids as reflected by plasma concentrations in cholesteryl esters and phospholipids.

C.2. Methods

C.2.1. Study Subjects

The Atherosclerosis Risk in Communities (ARIC) is a cohort study established in 1987. It was designed to investigate the etiology of atherosclerosis and its clinical sequelae. In each of four US communities--Forsyth County, NC, Jackson, MS, suburbs of Minneapolis, MN, and Washington County, MD—approximately 4,000 adults aged 45-64 years were recruited and invited to four examinations, three years apart (1987-89, 1990-92, 1993-95, 1996-98) or visits 1 through 4, respectively. Three out of the four cohorts represented the ethnic mix of their communities, while at Jackson, MS, only African-American residents were recruited (403).

Out of the total sample examined at baseline (N=15,792) we restricted these analyses to 11,557 individuals aged 50 years or older at baseline since research clearly shows that risk of cognitive decline in general, and of dementia in particular, is negligible prior to the age of 60 years (which is the age at which the youngest individuals in this cohort were re-examined in visit 4) (404). Eligibility for these analyses further required complete data on cognitive functioning at visits 2 (1990-92) and 4 (1996-98) and also complete plasma data at visit 1 (1987-89), which yielded n=2,251 men and women residing in the suburbs of Minneapolis, MN.

C.2.2. Cognitive Assessment

Three measures of cognitive functioning were made during visits 2 and 4 of the ARIC study, and these measures relied on the following instruments: Delayed Word Recall Test (DWRT) (167); the Digit Symbol Substitution portion of the Revised Wechsler Adult Intelligence Scale (DSST/WAIS-R) (405), and Word Fluency Test (WFT) of the Multilingual Aphasia Examination, also know as the controlled oral word association (170):

(1) *Delayed Word Recall Test (DWRT)*: This screening tool assesses verbal learning and recent memory. It requires from the respondent to recall 10 common words after a 5-minute interval during which another test is administered. Test scores may range between 0 and 10 words recalled and the time limit for recall is set at 60 seconds. The 6-months test-retest reliability of DWRT was previously shown to be high among 26 normal elderly individuals (Pearson correlation coefficient, r=0.75) (167).

(2) *Digit Symbol Substitution (DSST/WAIS-R):* This test is a paper-and-pencil test requiring timed translation of numbers 1 through 9 to symbols using a key. The test measures psychomotor performance and is relatively unaffected by intellectual ability, memory, or learning for most adults(170). It appears to be a sensitive and reliable marker of brain damage(407). The test score can range between 0 and 93 and it refelects the correctly translated number of digit-symbol pairs within a time limit of 90 seconds. Short-term test-retest reliability over 2-5 weeks has been found to be high in individuals aged 45-54 years (r=0.82) (405).

(3) *Word Fluency Test (WFT):* This test requires subjects to record as many words as possible using the letters F, A and S and to list these words, the subject is given only 60 seconds per letter. The total score corresponds to the total number of words generated during these three trials. The test is particularly sensitive to linguistic impairment (170, 408) and early mental decline in older persons (409). It is also a sensitive marker of damage in the left lateral frontal lobe (170, 408). The immediate test-retest correlation coefficient based on an alternate test form has been found to be high (r=0.82); (410).

Decline in the three separate areas of cognition was assessed. Cutoff points were determined for decline in each domain of cognition using the Reliable Change Index (RCI) method to correct for measurement error and practice effects (411).

RCI is defined as $((X_2-X_1)-(M_2-M_1))/S.E.D.$, where X_1 is the individual's score at baseline, X_2 the individual's score at follow-up, M_1 and M_2 are the group mean pretest and follow-up scores respectively, and S.E.D. is the observed standard error of the difference scores. Scoring below an RCI of -1.645 was regarded as a "statistically reliable" deterioration in the test scores. A composite measure of the three RCIs to assess global cognitive decline (GCD) was created using principal components analysis (PCA). Similarly, the cutoff point chosen corresponded to a composite score of reliable decline below -1.645. Multivariate analysis included control for the baseline cognitive score in its continuous form (assessed at visit 2) on the particular instrument. Models with GCD as the main outcome controlled for a measure of global baseline cognitive functioning (GBCF) which reduces the three baseline scores into a single component using PCA.

C.2.3. Plasma fatty acid exposures

Twelve-hour fasting blood was collected according to the ARIC study wide protocol. The Minneapolis field center conducted fatty acid analysis in plasma phospholipid and cholesteryl ester fractions on visit 1 blood specimens. The procedure is described in detail elsewhere (371). The identity of 28 fatty acid peaks were revealed by gas chromatography by comparing each peak's retention time to the retention times of fatty acids in synthetic standards of known compositions. The relative amount of each fatty acid (as a percent of all fatty acids) was computed through integration of the area under the peak and division of the result by the total area for all fatty acids (×100). Data from the chromatogram were transferred electronically to a computer for analysis. Fatty acids are expressed as % of total in each fraction. Although all groups of fatty acids were assessed in relation to global cognitive decline (SFA, MUFA, LA, LNA and ω-3 and ω-6 HUFAs) one main exposure of interest was considered for domain-specific and interaction analysis namely EPA+DHA (20:5+22:6ω-3) which were also the ω-3 HUFAs.

Test-retest reliability coefficients (individuals sampled 3 times, 2 weeks apart) for various plasma fatty acids ranged from 0.50 to 0.93 for cholesteryl esters to 0.89 for phospholipids(423).

C.2.4. Covariates

Most covariates considered were measured at visits 1 or 2, although some were defined according to measurements that spanned all four visits. Age, gender, and education were all self-reported. Behavioral factors measured at visit 1 included smoking, alcohol and caffeine consumption as well as physical activity (424). A validated index of physical activity was derived at visit 1, summing sports, work and leisure indices which ranged from a score of 1 (low) to 5 (high) (425). Body mass index at visit 1 was computed by dividing weight (in kilograms) by height-squared (in square meters). Baseline dietary intake of antioxidants and other micronutrients (mainly Vitamins B_6 , B_{12} and folate) was considered as well. Usual dietary intake of these nutrients was estimated from an interviewer-administered semi-quantitative food frequency questionnaire (FFQ) modified from a 61-item questionnaire developed and validated by W. Willet and colleagues against multiple food records among a sub-sample of the Nurses' Health Study cohort. Results of the validation study suggested that for all nutrients considered there was only up to 3% extreme quintile misclassification and that overlap between the upper two quintiles and lower two quintiles between the two methods was >70% (416). Daily intake of nutrients has been calculated by multiplying the nutrient content of each food in the portion specified by the frequency of daily consumption and summing the results. The nutrient content of each food was obtained from the Harvard nutrient data base for which the primary source was the Department of Agriculture handbook (417).

All these covariates were considered as potential confounders in the multivariate analysis. Potential effect modifiers were grouped as genetic (presence of ApoE ε4 allele) and co-morbid conditions. To measure hypertensive status, blood pressure levels were calculated as the average of the second and third of three consecutive measurements with a random-zero sphygmomanometer. The cutoff point often used for hypertension is ≥ 140 mm Hg. for systolic blood pressure (SBP) and ≥ 90 mm Hg. for diastolic blood pressure (DBP). Hypertensive individuals were defined as those who screened positive for measured hypertension at either visits 1 through 4 or were taking anti-hypertensive medication over the past two weeks prior to examination on any of those visits. Other conditions included stroke or TIAs, type II diabetes mellitus (defined as fasting blood glucose ≥ 140 mg./dl or self-reported diabetes or use glucose lowering medication) and dyslipidemia (fasting blood HDL-C<40 (men) or <50 (women) and triacylglycerol level greater than 150 mg/dl as recommended by NCEP (426)) at any visit, hypercoagulable profile (upper quintile of at least two of: fibrinogen, vWF and factor VIII), poor pulmonary function (ratio $FEV_1/FVC \leq 0.70$ as measured by a spirometer at visits 1 or 2), and depressive symptoms at visit 2 using 21-item vital exhaustion scale (427, 428). A binary cutoff point for depression was chosen based on the 80^{th} percentile of the scale scores.

C.2.5. Statistical Analyses

We carried out univariate analyses of predictor and outcome variables as well as covariates. For bivariate analyses of exposure and outcome, we computed means of predictor variables across outcome groups (0: no decline; 1: declined) and assessed statistical significance of differences using independent samples *t*-test for continuous and χ^2 test for categorical predictors at an alpha level of 0.05. We computed odds ratios of decline for each increase in exposure by 1 SD by conducting multivariate logistic regression analysis.

Control for confounding was done using backward elimination, retaining in the model those variables which changed the estimated effect (odds ratio) of the exposure by more than 5% were retained in the final model. This level is more suitable than 10% since sensitivity of odds ratios to confounding effects tends to increase with increase in sample size (458). We used likelihood ratio tests to assess statistical significance of interaction between exposure and potential effect modifiers at a type I error level of 0.20, after obtaining the final parsimonious model. The multivariate models can be summarized by equations **(5.10.1.)** and **(5.10.2.)**:

$$\text{Logit}[\Pr(Y=1 \mid B, Z)] = \beta_0 + \beta_1 B_1 + \Sigma\beta_j Z_j \qquad \textbf{(5.10.1.)}$$

$$\text{Logit}[\Pr(Y=1 \mid B, Z, M)] = \beta_0 + \beta_1 B_1 + \beta_3 M + \Sigma\beta_j Z_j + \gamma B_1 \times M \qquad \textbf{(5.10.2.)}$$

Where Logit is the $\text{Log}_e[\Pr/(1-\Pr)]$, Y is the binary outcome of cognitive decline, B_1 is the main exposure of interest as derived from cholesteryl esters or phospholipids fatty acids, Z_j is a vector of potential confounders, and M is the effect modifier. Statistical analyses were conducted using STATA ver. 8.2 (412).

C.3. Results

C.3.1.Baseline Characteristics

A total of 2,251 study subjects had complete plasma analysis at visit 1 and cognitive decline data between visits 2 and 4. They were all white men and women residing in the suburbs of Minneapolis (one of the ARIC study centers). Looking at distribution of characteristics of this sample of subjects by cognitive status, those who did not decline differed from those who did on several socio-demographic, behavioral and health-related factors. Specifically, they were on average younger, more physically active, reported less depressive symptoms and had a relatively hypocoagulable profile.

Those who declined compared to those who did not revealed also a greater baseline cognitive score. Furthermore, their mean cognitive change between the two visits was significantly larger in absolute value (Table 5.3.1.).

C.3.2.Plasma fatty acids and global cognitive decline

Table 5.3.2. shows the mean plasma concentration of individual as well as families of fatty acids across global cognitive decline categories. Within the cholesteryl ester fraction, those who declined had higher concentrations of palmitic and arachidonic acids, and lower concentration of linoleic acid. After controlling for other potentially confounding factors including other fatty acids, total PUFAs, total ω-6 PUFAs and linoleic acid were all inversely related to decline, while greater arachidonic acid remained a significant risk factor for decline. In the plasma phospholipid fraction, the unadjusted means revealed the same pattern across cognitive status. However, adjusted odds ratios of decline with each SD increment in these fatty acids did not differ significantly from the null value of 1, with the exception of palmitic acid (a saturated fatty acid) which seemed consistently associated positively with the risk of cognitive decline. Hence, ω-3 PUFAs in general and DHA+EPA in particular had no significant effect on global cognitive decline.

C.3.3. ω-3 fatty acids and cognition: a subgroup analysis

Table 5.3.3. shows a set of multivariate logistic models of plasma ω-3 HUFAs (DHA+EPA) on cognitive decline in the three areas of interest as well as globally for both the cholesteryl ester and phospholipid fractions. Covariates considered as potential confounders included baseline cognitive score, socio-demographic, behavioral and nutritional factors (including other groups of fatty acids in that particular fraction). In contrast to what was noted in Table 2, Table 3 shows that the greater DHA+EPA was associated with less decline in verbal fluency (WFT) for both cholesteryl ester and phospholipid fractions (Odds ratios were: 0.74 (0.57, 0.97) and 0.73 (0.58, 0.93), respectively).

Subgroup analysis by selected cardiovascular, genetic and other health conditions that have been associated with increased oxidative stress indicated that some of these factors may modify the effect of DHA+EPA on the main outcomes of interest. In particular, when the outcome was decline in verbal fluency (WFT), DHA+EPA was mostly protective among hypertensive and dyslipidemic subjects and subjects with a lower score on depressive symptoms. This significant interaction (based on likelihood ratio test at type I error 0.20) was revealed for both cholesteryl esters and phospholipids plasma fractions. In addition, for the phospholipids fraction, an interaction was found with pulmonary function, whereby subjects with poor function had stronger protective effect of increased level of DHA+EPA on decline in verbal fluency. For all other domains of cognition (DWRT and DSST/WAIS-R), there were weak to null effects of DHA+EPA on cognitive decline in both fraction (cholesteryl esters and phospholipids), overall and across subgroups.

C.4. Discussion

This is one of the very few published reports on the association of plasma fatty acids with cognitive decline among older adults using a prospective cohort design. Our findings indicated global cognitive decline was affected by a greater relative amount of palmitate (a saturated fatty acid) in both fractions. In cholesteryl esters, risk of global cognitive decline was increased by a greater arachidonic acid concentration (an ω-6 highly unsaturated fatty acid) and a lower amount of linoleic acid (an ω-6 medium chain PUFA), even after controlling for several sociodemographic, behavioral and nutritional factors. More importantly, lower concentrations of our main exposure of interest -- ω-3 highly unsaturated fatty acids (mainly DHA and EPA) -- was associated with a higher risk of decline in verbal fluency, particularly among hypertensive and dyslipidemic subjects, as well as the less depressed individuals at baseline. These interactions were consistent between cholesteryl ester and phospholipid fractions of plasma with few exceptions.

No effect was observed on delayed word recall (DWRT) or psychomotor speed (through DSST/WAIS-R test) among any of the subgroups considered.

Our study included a group of white middle-aged men and women residing in the suburbs of Minneapolis. They were in general highly educated with very few having less than a high school education. Since education tends to affect most neuropsychological tests, having a relatively homogenous group is an advantage despite the fact that we are able to control for educational level in the analysis. Average changes in cognitive scores over a period of six years were relatively small when looking at the range of values that each test can take. One main advantage of this study over previous ones is the use of the reliable change index (RCI) in order to assess the degree of change in cognitive scores between two points in time and choose the cutoff point for each domain that corresponds to a statistically reliable decline. The cut-point chosen was an RCI<-1.645. This means that the level of sensitivity chosen was 90%, with the lower 5% of the distribution having declined while the upper 5% would have improved significantly. The RCI method corrects for measurement error and practice effects, by taking into account the difference in group means of pretest and posttest scores. Hence, the choice of the cutoff point, rather than being arbitrary, is one that is supported by neuropsychological evidence(411).

The study had a few limitations. First, it made use of tissue composition of fatty acids which does not necessarily reflect the proportion of fatty acids in the diet. Nevertheless, ARIC had also collected dietary intake data using a semi-quantitative food frequency questionnaire at the same baseline visit (i.e. visit 1). A study by Ma and colleagues had shown that intake of DHA and EPA was highly correlated with their concentrations in both cholesteryl esters and phospholipids (371). In addition, repeated measures of a sub-sample of plasma specimens indicated that fatty acid concentration in both fractions is reliable over the short and long term (423).

Previous studies have also indicated that plasma levels of highly unsaturated fatty acids may also be good reflection of long-term dietary intake (372-375, 463). However, the main advantage of using a biomarker rather than a self-report of intake is that we are certain that errors in the exposure are independent of errors in our outcome measure. This would in most cases lead to an attenuation of effect in the presence of measurement error and hence lead to an odds ratio that is biased towards the null value of 1. Another limitation is that the sum of fatty acids in each fraction is 100. Therefore, a higher percentage of a specific group (e.g. SFA) will automatically reflect a lower percentage of another. Hence, there is a problem of interdependence which makes it difficult to interpret the effect of a single constituent or group of constituents. We are also unable to translate these findings quantitatively into dietary recommendations (e.g. number of servings of fish per week). Finally, a prior analysis indicated that there was a marked differential in short-term reliability between fatty acids that were major constituents (reliability coefficient > 0.65) and those that had a percentage less than 1% of total fatty acids(423).

Previous observational studies suggested that the biochemical composition of blood components in terms of fatty acids differs significantly between subjects with normal cognitive functioning and patients with some form of cognitive impairment. A study Conquer and colleagues (384) found that patients with Alzheimer's disease or other dementia had lower plasma phosphatidyl choline (PC) level of ω-3 fatty acids which include EPA and DHA as well as a lower ratio of ω-3/ω-6 fatty acids when compared to normal controls. The authors concluded that a decreased plasma level of ω-3 fatty acids, and in particular DHA, is associated with Alzheimer's disease as well as other forms of cognitive impairment. Another case-control study conducted by Tully and colleagues (385) showed that patients with Alzheimer's disease had significantly lower levels of serum cholesteryl ester-eicosapentaenoic acid (EPA) as compared to the controls.

A third recent study adopted a nested case-control design within the prospective Epidemiology of Vascular Aging cohort. Its main aims were to assess fatty acid composition of erythrocyte membranes as a risk factor for cognitive decline (2-point decline or more in MMSE) among 246 men and women aged 63-74 years within the 4-year follow-up which was conducted in France. The study found that total ω-6 polyunsaturated fatty acids were associated with a greater risk of cognitive decline with an odds ratio of 1.59 (95% CI: 1.04, 2.44). Conversely, a higher proportion of total ω -3 fatty acids were associated with a lower risk of cognitive decline, with an odds ratio of 0.59 (95% CI: 0.38, 0.93). Hence, overall there was an inverse association between cognitive decline and the ratio of ω-3/ ω-6 fatty acids in erythrocytes (386). While the majority of these studies showed an inverse association of plasma and erythrocyte ω -3 fatty acids with cognition among older adults, others found either a null association or an opposite relationship. In fact, a cross-sectional study – the Canadian Study of Health and Ageing – showed that the mean relative plasma concentration of ω-3 fatty acids as well as total polyunsaturated fatty acids was higher among subjects aged 65 years or more with cognitive impairment or dementia after controlling for age, sex, education, smoking, alcohol intake, body mass index, history of cardiovascular disease, and apolipoprotein E ε4 genotype (387).

Our hypothesized effect of this class of fatty acids on cognition has been linked with several biologically plausible mechanisms. These include preventing vascular abnormalities (464) , reducing inflammation (465) or both, as well as their impact on membrane fluidity and ultimately neurotransmission (29). For example, excess ω-3 HUFAs (mainly DHA and EPA) can reduce the risk of thrombosis and reduce blood pressure, while both conditions are thought to alter arterial walls and impair oxygen and nutrient supplementation needed for normal cerebral functioning (466, 467).

It has also been established that ω-3 HUFAs can reduce the level of plasma triacylglycerols (345, 346) and improve glycemic control and insulin sensitivity in type II diabetes (340, 341). Their ability to lower LDL-C has also been shown (349) although this effect was not necessarily specific to this class of fatty acid, but rather to all polyunsaturated fatty acids, including linoleic acid.

Another suggested paradigm is that DHA and EPA exert anti-inflammatory influences by suppressing the metabolism of arachidonic acid (an ω-6 HUFAs) into proinflammatory cytokines. In fact, the enzyme phospholipase (PLA_2) can release specific fatty acids from the sn-2 position of membrane phospholipids, but with markedly differing consequences. Further, dihomogammalinolenic acid (DGLA ~ 20:3 ω -6), arachidonic acid (AA ~ 20:4 ω -6) and eicosapentaenoic acid (EPA ~ 20:5 ω -3) can be transformed into prostaglandins (PGl) of the 1-, 2-, and 3-class, respectively. While the PGl_2 is highly pro-inflammatory, PGl_3 is anti-inflammatory and PGl_1 was shown to have intermediate properties. It has been hypothesized that a highly reactive PLA_2 is found in various brain disorders, including cognitive impairment (35). This high reactivity, when coupled with an elevated concentration of ω-6 fatty acids in brain membranes would lead to aggravated inflammatory conditions and development of neuronal dysfunction manifesting itself in psychiatric and cognitive disorders.

A third mechanism may involve the biophysical properties of brain membranes and how they are affected by the ratio of ω-3 to ω-6 fatty acids. Yehuda and colleagues (37) proposed a unifying model that involves the hypothalamic-pituitary-adrenal axis.

According to this model, essential fatty acids are involved in neurotransmitters in the brain and hypothalamus, in stimulating corticosteroid releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), and in the production of cortisol from cholesterol by the enzyme P450. P450 is involved in dopamine (the first molecule in the axis) and cortisol production (which is the end product of the axis) and thereby accounts for the ability of cortisol in the blood to affect cognitive function. However, for this axis to actually produce the end product "cortisol", cholesterol must be bioavailable in the brain, a state that is highly dependent on membrane fluidity and hence on the ratio of ω-3 to ω-6 fatty acids.

Based on our findings, there is reason to believe that subjects who are under increased oxidative stress, particularly hypertensive and dyslipidemic subjects, may benefit by enriching their diet with ω-3 HUFAs, which are mostly found in cold water fish (e.g. salmon, tuna, mackerel) and other foods of marine origin. Future research should attempt to conduct subgroup analysis using actual markers of oxidative stress (468). In addition, a randomized trial may be warranted comparing subjects with varying levels of oxidative stress in terms of their cognitive change response to increasing dietary intake of ω-3 HUFAs using a more comprehensive battery of neuropsychological tests.

TABLE 5.3.1.
Characteristics of study subjects with complete cognitive and plasma data by global cognitive decline status (N=2,251) ; ARIC 1987-98

Characteristic	GCD (RCI<-1.645) No decline (N=2,111)	Decline (N=140)	All (N=2,251)
Female	50.69	50.70	50.69
Age (years)[1]	56.21±4.22**	57.74±4.22	56.30±4.24
Education[1]			
Incomplete high school	6.78	5.00	6.67
High School	36.40	32.86	36.18
> High School	56.82	62.14	57.16
Apo E ε4 allele[1]	28.74	30.37	28.84
Smoking status[1]			
Never smoker	40.38	40.71	40.40
Former smoker	41.94	40.0	41.82
Current smoker	17.68	19.28	17.78
Alcohol (g/day)[1]	8.01±13.48	9.04±13.47	8.08±13.47
Caffeine (mg/day)[1]	350.59±326.82	310±311	348.08±325.93
Physical activity scale[1]	7.34±1.32**	7.09±1.39	7.33±1.33
Body mass index (kg/m^2)[1]	27.21±4.41	26.60±4.33	27.17±4.41
Total energy intake (Kcal/day)[1]	1583±559	1546±568	1581±559
Vitamin A (in 1000 IUs/day)[1]	8.65±6.90	8.63±5.82	8.65±6.83
Vitamin B_6 (mg/day)[1]	1.75±0.67	1.74±0.64	1.74±0.66
Vitamin B_{12} (mcg/day)[1]	7.06±3.47	7.01±3.97	7.06±3.50
Vitamin C (mg/day)[1]	112.80±70.78	110.66±56.16	112.67±69.95
Vitamin E (mg/day)[1]	4.66±3.02	4.66±2.99	4.66±3.01
Folate (mcg/day)[1]	218.80±95.21	213.80±91.48	218.48±94.97
Stroke/TIAs[2]	9.00	13.6	9.28
Hypertensive[2]	48.93	54.58	49.27
Dyslipidemia[2]	37.38	32.14	37.05
Type II Diabetes[2]	13.64	17.14	13.86
Depression scale[3]	8.08±7.03**	9.73±8.25	8.19±7.12
Poor pulmonary function (FEV_1/FVC <70)[3]	18.82	20.71	18.93
Hypercoagulable profile[1]	10.75**	16.43	11.11
Fibrinogen value	293.87±59.95	302.91±74.32	294.44±60.96
vWF	110.71±39.50**	117.75±41.77	111.14±39.67
Factor VIII	124.00±32.33	128.29±37.63	124.28±32.70
Baseline cognitive scores (V2)[3]			
DWRT	6.75±1.43**	7.29±1.32	6.78±1.43
DSST/WAIS-R	51.58±10.10	52.76±0.91	51.65±10.15
WFT	37.05±11.28**	45.66±12.09	37.59±11.52
Cognitive Decline (V4-V2) [3]			
DWRT	-0.10±1.42**	-1.94±1.62	-0.21±1.50
DSST/WAIS-R	-3.88±5.79**	-13.1±8.06	-4.45±6.36
WFT	-1.12±7.13**	-12.77±8.91	-1.84±7.78

**p<0.05 for null hypothesis that means or proportions are equal between decline and no decline groups;
[1] Covariate measured at visit 1; [2] Covariate measured at visits 1 through 4. [3] Covariate with other time frame.
[4] $\bar{x} \pm SD$.

TABLE 5.3.2.
Mean ±*SD* plasma concentrations of fatty acid groups by cognitive decline status and adjusted odds ratios[2] (ORs) for decline in global cognitive functioning by change in fatty acid concentration (N=2,251); ARIC 1987-98[1]

Fatty acid	GCD (RCI<-1.645) No decline (N=2,111)	Decline (N=140)	All (N=2,251)	OR (95% CI) per SD difference
Plasma Cholesteryl esters				
Total SFAs	17.93±1.98	18.16±1.93	17.95±1.98	1.11 (0.95, 1.31)
Stearic acid (18:0)	0.89±0.19	0.86±0.15	0.89±0.19	0.85 (0.70, 1.03)
Palmitic acid (16:0)	10.02±0.79**	10.22±0.83	10.03±0.79	1.28 (1.07, 1.54)**
Total MUFAs	15.91±1.96	16.04±2.04	15.92±1.97	0.69 (0.46, 1.02)
Oleic acid (18:1ω-9)	15.83±1.95	15.96±2.03	15.84±1.96	0.70 (0.47, 1.04)
Total PUFAs	65.86±3.73	65.26±3.86	65.82±3.74	0.55 (0.37, 0,81)**
Total ω-6 PUFAs	64.39±3.82	63.76±3.92	64.35±3.83	0.54 (0.36, 0.82)**
AA (20:4 ω -6)	8.27±1.67**	8.72±1.77	8.30±1.68	1.21 (1.00, 1.47)**
Linoleic acid (18:2 ω -6)	54.26±4.64**	53.13±4.91	54.20±4.67	0.64 (0.49, 0.83)**
Total ω-3 PUFAs	1.42±0.43	1.46±0.39	1.42±0.43	1.08 (0.92, 1.26)
EPA (20:5ω-3)	0.55±0.29	0.57±0.25	0.55±0.29	0.84 (0.66, 1.05)
DHA (22:6ω-3)	0.45±0.16	0.47±0.17	0.45±0.16	1.18 (0.97, 1.44)
Linolenic acid (18:3 ω-3)	0.41±0.10	0.41±0.09	0.41±0.10	0.97 (0.82, 1.16)
DHA+EPA	1.01±0.40	1.04±0.36	1.01±0.39	1.09 (0.94, 1.27)
(DHA+EPA)/AA	0.12±0.05	0.12±0.04	0.12±0.05	0.96 (0.80, 1.15)
Plasma phospholipids				
Total SFAs	49.36±2.95	49.31±2.75	49.36±2.95	0.98 (0.82, 1.16)
Stearic acid (18:0)	13.31±1.18	13.15±1.31	13.30±1.19	1.04 (0.84, 1.29)
Palmitic acid (16:0)	25.36±1.61**	25.72±1.88	25.38±1.63	1.24 (1.05, 1.47)**
Total MUFAs	9.23±1.09	9.22±1.13	9.23±1.09	0.97 (0.82, 1.16)
Oleic acid (18:1ω-9)	8.50±1.09	8.50±1.15	8.51±1.10	1.00 (0.84, 1.18)
Total PUFAs	41.91±1.67	41.92±1.65	41.91±1.67	0.99 (0.84, 1.17)
Total ω-6 PUFAs	38.20±1.78	38.08±1.74	38.20±1.78	0.93 (0.79, 1.10)
AA (20:4 ω -6)	11.47±1.93**	11.92±1.98	11.50±1.94	1.16 (0.93, 1.43)
Linoleic acid (18:2 ω -6)	21.96±2.60**	21.37±2.68	21.93±2.61	0.87 (0.70, 1.09)
Total ω-3 PUFAs	3.59±1.05	3.71±1.07	3.59±1.05	1.11 (0.95, 1.30)
EPA (20:5ω-3)	0.57±0.31	0.57±0.25	0.57±0.31	0.96 (0.80, 1.16)
DHA (22:6ω-3)	2.87±0.88	2.98±0.94	2.88±0.88	1.13 (0.96, 1.32)
Linolenic acid (18:3 ω-3)	0.14±0.05	0.14±0.04	0.14±0.05	1.03 (0.87, 1.22)
DHA+EPA	3.44±1.05	3.56±1.07	3.44±1.05	1.11 (0.95, 1.29)
(DHA+EPA)/AA	0.31±0.11	0.31±0.11	0.31±0.11	0.99 (0.83, 1.18)

**$p<0.05$ for null hypothesis that means are equal between decline and no decline groups;
[1] SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; ω-3HUFAs, omega-3 highly unsaturated fatty acids (EPA+DHA); AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
[2] Each fatty acid exposure was included in a separate multivariate logistic model. Covariates considered as potential confounders were: baseline cognitive functioning, socio-demographics (age, sex, education); behavioral factors (smoking, alcohol and caffeine consumption and physical activity) and nutritional factors (other fatty acid groups in the fraction, body mass index, caloric intake, intake of antioxidants and vitamins B_6, B_{12} and folate). Control for confounding was done using backward elimination, retaining in the model those variables which changed the estimated effect (odds ratio) of the exposure by more than 5% were retained in the final model.

TABLE 5.3.3.
Multivariate logistic regression[1] of plasma ω-3 HUFAs (EPA+DHA) on cognitive decline: a subgroup analysis; ARIC 1987-98

	Cognitive decline (RCI<-1.64)							
	DWRT		DSST/WAIS-R		WFT		GCD	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Plasma Cholesteryl esters								
All subjects	1.02	(0.85, 1.21)	1.00	(0.83, 1.20)	0.74	(0.57, 0.97)*	1.09	(0.94, 1.27)
Normotensive	1.07	(0.85, 1.36)	1.07	(0.84, 1.36)	0.99	(0.71, 1.38)	1.16	(0.95, 1.41)
Hypertensive	0.94	(0.72, 1.23)	0.90	(0.67, 1.21)	0.55	(0.36, 0.84)***	1.05	(0.81, 1.36)
ApoEε4 (No allele)	1.05	(0.84, 1.31)	0.97	(0.76, 1.25)	0.61	(0.43, 0.87)*	1.08	(0.90, 1.30)
ApoEε4 (1 or 2 alleles)	0.99	(0.72, 1.35)	1.10	(0.82, 1.46)	0.80	(0.45, 1.36)	1.01	(0.90, 1.30)
Normal lipid profile	0.94	(0.73, 1.20)	1.04	(0.83, 1.29)	0.87	(0.66, 1.15)	1.10	(0.93, 1.30)
Dyslipidemia	1.16	(0.87, 1.53)	0.92	(0.66, 1.28)	0.49	(0.28, 0.87)**	1.05	(0.76, 1.44)
No Stroke/TIAs	1.02	(0.85, 1.23)	1.02	(0.84, 1.23)	0.74	(0.55, 1.00)*	1.10	(0.94, 1.29)
Stroke/TIAs	0.96	(0.46, 1.99)	0.86	(0.44, 1.66)	0.72	(0.38, 1.38)	0.95	(0.58, 1.55)
Non-diabetic	1.00	(0.81, 1.22)	0.99	(0.80, 1.24)	0.79	(0.59, 1.05)	1.13	(0.96, 1.34)
Type II diabetes	1.09	(0.79, 1.49)	0.96	(0.67, 1.38)	0.65	(0.32, 1.33)	0.93	(0.61, 1.42)
Hypocoagulable profile	1.02	(0.85, 1.22)	1.00	(0.82, 1.22)	0.79	(0.59, 1.05)	1.13	(0.97, 1.32)
Hypercoagulable profile	0.98	(0.53, 1.82)	1.08	(0.65, 1.80)	0.55	(0.27, 1.11)	0.83	(0.48, 1.43)
Depressive symptoms (≤10)	1.03	(0.85, 1.27)	1.03	(0.83, 1.27)	0.62	(0.43, 0.88)*	1.13	(0.95, 1.35)
Depressive symptoms (>10)	0.99	(0.71, 1.39)	0.95	(0.65, 1.37)	0.97	(0.73, 1.31)**	0.99	(0.74, 1.34)
Good pulmonary function	1.07	(0.88, 1.29)	0.96	(0.77, 1.20)	0.77	(0.66, 1.04)	1.07	(0.89, 1.28)
Poor pulmonary function	0.86	(0.56, 1.31)	1.13	(0.82, 1.54)	0.61	(0.31, 1.20)	1.16	(0.88, 1.53)
Plasma phospholipids								
All subjects	0.87	(0.71, 1.06)	1.02	(0.84, 1.24)	0.73	(0.58, 0.93)*	1.11	(0.95, 1.29)
Normotensive	0.98	(0.73, 1.32)	1.04	(0.78, 1.38)	0.89	(0.65, 1.23)	1.18	(0.95, 1.48)
Hypertensive	0.78	(0.59,1.03)	0.97	(0.74, 1.28)	0.64	(0.44, 0.91)**	1.06	(0.84, 1.33)
ApoEε4 (No allele)	0.95	(0.73, 1.23)	0.97	(0.75, 1.25)	0.68	(0.51, 0.91)*	1.14	(0.95, 1.38)
ApoEε4 (1 or 2 alleles)	0.77	(0.55, 1.08)	1.18	(0.85, 1.64)	0.61	(0.35, 1.08)	0.86	(0.61, 1.22)
Normal lipid profile	0.77	(0.59, 1.01)	0.99	(0.78, 1.26)	0.85	(0.66, 1.10)	1.13	(0.94, 1.35)
Dyslipidemia	1.06	(0.77, 1.46)	1.04	(0.74, 1.44)	0.49	(0.30, 0.84)**	1.09	(0.80, 1.49)
No Stroke/TIAs	0.87	(0.71, 1.07)	1.02	(0.83, 1.25)	0.74	(0.57, 0.96)*	1.10	(0.93, 1.30)
Stroke/TIAs	0.91	(0.44, 1.84)	1.00	(0.57, 1.76)	0.61	(0.34, 1.11)	1.10	(0.72, 1.68)
Non-diabetic	0.85	(0.69, 1.06)	1.01	(0.81, 1.27)	0.75	(0.58, 0.97)*	1.17	(0.99, 1.38)
Type II diabetes	0.92	(0.60, 1.40)	0.99	(0.66, 1.46)	0.73	(0.37, 1.43)	0.90	(0.57, 1.42)
Hypocoagulable profile	0.87	(0.71, 1.07)	0.97	(0.79, 1.19)	0.74	(0.58, 0.96)*	1.10	(0.95, 1.31)
Hypercoagulable profile	0.83	(0.44, 1.56)	1.58	(0.89, 2.80)	0.69	(0.30, 1.23)	1.19	(0.77, 1.86)
Depressive symptoms (≤10)	0.80	(0.63, 1.02)	1.01	(0.81, 1.27)	0.62	(0.46, 0.84)*	1.08	(0.89, 1.32)
Depressive symptoms (>10)	1.04	(0.74, 1.45)	1.01	(0.69, 1.48)	1.00	(0.71, 1.39)**	1.18	(0.91, 1.52)
Good pulmonary function	0.89	(0.72, 1.12)	0.96	(0.77, 1.20)	0.79	(0.61, 1.02)	1.09	(0.91, 1.31)
Poor pulmonary function	0.78	(0.50, 1.20)	1.21	(0.83, 1.78)	0.52	(0.28, 0.96)**	1.17	(0.86, 1.59)

* $p<0.05$ for testing the null hypothesis that $\beta_1=0$. See equations (1) and (2); **p<0.20 for testing the null hypotheses that $\gamma=0$ using the likelihood ratio test. See equation (5.10.2).; ***p<0.05 for testing the null hypotheses that $\gamma=0$ using the likelihood ratio test, to allow for multiple comparisons. ω-3HUFAs, omega-3 highly unsaturated fatty acids (EPA+DHA); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; [2] Control for confounding was done using backward elimination, retaining in the model those variables which changed the estimated effect (odds ratio) of the exposure by more than 5% were retained in the final model. Covariates considered as potential confounders were: baseline cognitive functioning, socio-demographics (age, sex, education); behavioral factors (smoking, alcohol and caffeine consumption and physical activity) and nutritional factors (other fatty acid groups in the fraction, body mass index, caloric intake, intake of antioxidants and vitamins B_6, B_{12} and folate).

C h a p t e r 6

CONCLUSIONS

A. Recapitulation of overall study aims, findings and degree to which the goals of the doctoral research have been met

In summary, my doctoral research had three study aims that were translated into three research papers. All aims were covered and hypotheses listed in Chapter III were tested.

The first paper was purely methodological in nature. It aimed at validating a FFQ for the estimation of essential fatty acid intake and biologically plausible combinations and ratios of these nutrients. The study utilizes two reference measures: (i) an alloyed gold standard (*T**) based on a biomarker of specific fatty acid levels in plasma phospholipids and empirically derived biochemical equations that estimate true dietary intake (381-383). This alloyed gold standard takes the biochemical approach to validation (ii) Two instrumental biomarkers for the level of fatty acids in the cholesteryl ester (*M*) and phospholipids (*N*) fractions of plasma which were previously shown to be linearly related to dietary intake as assessed by more reliable reference methods such as multiple 24-hour recalls, food records or diet history (373-375, 436-440). These instrumental biomarkers take an epidemiological approach to validation. Findings from this study and its overall methodology may be used in subsequent analyses to adjust for measurement error in causal models linking intake of essential fatty acids and their biologically plausible ratios with disease outcomes. The main parameters that were estimated in this paper were the attenuation factor for each fatty acid exposure and the error variance covariance matrix.

These can be used in regression calibration and simulation extrapolation analysis using the same food frequency questionnaire administered to a comparable population.

My second paper was a prospective study which assessed the effect of low ω-3 fatty acid status on six-year cognitive decline in men and women aged 50 years and older, as well as the interaction of this risk factor with elevated blood pressure. The study showed that increased dietary intake of long chain ω-3 fatty acids and balancing long-chain ω-3/ω-6 decreased the risk of cognitive decline in verbal fluency, particularly among hypertensive subjects. This finding also held for the corresponding plasma analytes in the cholesteryl ester and phospholipids fractions.

My third paper looked at the effect of plasma fatty acids on cognitive decline among older adults using a prospective cohort design, expanding the effect to various fatty acid types and interaction with a number of oxidative-stress inducing conditions. Findings indicated that global cognitive decline was affected by excess amount of palmitate (a saturated fatty acid) in both fractions. In cholesteryl esters, risk of global cognitive decline was increased by an elevated arachidonic acid concentration (an ω-6 highly unsaturated fatty acid) and a lower amount of linoleic acid (an ω-6 medium chain PUFA), even after control was done on several sociodemographic, behavioral and nutritional factors. More importantly, lower concentrations of ω-3 highly unsaturated fatty acids (mainly DHA and EPA) were associated with a higher risk of cognitive decline in verbal fluency, particularly among hypertensive and dyslipidemic subjects, as well as the less depressed individuals at baseline. These interactions were consistent between cholesteryl ester and phospholipid fractions of plasma. No effects were observed on delayed word recall (DWRT) and psychomotor speed (through DSST/WAIS-R test) among any of the subgroups considered.

B. Strengths

The validation study (paper 1) is one of the very few attempts to estimate an error variance-covariance matrix ($\hat{\Sigma}_{uu}$) that can be used subsequently by other researchers for the purpose of correcting for measurement error in multivariate generalized linear models. Estimates of attenuation factors (λ_j) which can be used mainly in bivariate generalized linear models were also reported. The approach used was similar to previous research (429, 451, 452). While this article focused on regression calibration, other measurement error models utilize $\hat{\Sigma}_{uu}$, including simulation extrapolation (SIMEX), methods with instrumental variables and maximum-likelihood methods(447). Health outcomes that have traditionally been of interest in relation to essential fatty acids and the balance between them include coronary heart disease (324, 325), stroke (327, 328), type II diabetes (341), breast and prostate cancer (453, 454), depression (351, 352), cognitive functioning (137, 141, 388, 389), hypercoagulable profile (332, 336) and COPD (422).

One of the main strengths of the second paper is the prospective design used which thus far is unique in the literature testing this particular hypothesis (459). In addition, an evidence-based report suggested a need to look for the effect of ω-3 fatty acids on cognitive decline by cardiovascular disease status and to define exposure in terms of absolute value of medium chain and long chain fatty acids, as well as the ratio between ω-3 and ω-6 fatty acids in diet and plasma (460). All these suggestions were implemented in the present study. Moreover, this is the first study to assess effect modification by hypertensive status and to test at the population level a biological interaction documented in animal experimental work. Measurement error, which almost always accompanies dietary assessment, was corrected for in this study using regression calibration and SIMEX.

Another strength that is shared with the third paper is the use of the reliable change index (RCI) in order to assess the degree of change in cognitive scores between two points in time and choose the cutoff point for each domain that corresponds to a statistically reliable decline. The cut-point chosen was an RCI<-1.645. This means that the level of sensitivity chosen was 90%, with the lower 5% of the distribution having declined while the upper 5% would have improved significantly. The RCI method corrects for measurement error and practice effects, by taking into account the difference in group means of pretest and posttest scores. Hence, the choice of the cutoff point, rather than being arbitrary, is one that is supported by neuropsychological evidence.

An advantage of using biomarkers of dietary intake rather than self-reported intake is that we are certain that errors in the exposure are independent of errors in our outcome measure. This would in most cases lead to an attenuation of effect in the presence of measurement error and hence lead to an odds ratio that is biased towards the null value of 1.

C. Limitations

Some of the main limitations of the validation study include the lack of a reference method that is known to be more reliable than FFQs in the ARIC study (e.g. multiple 24-hour recalls or food records). However, because of correlated errors between self-report methods, the use of biomarkers has often been cited as a more adequate means to assess the extent of measurement error in a test instrument. Another drawback is the fact that plasma levels of fatty acids in both fractions studied constitute a short-term measure of intake although they have been shown to correlate well with long-term intake (9).

In addition, the lack of certainty as to the nature of the relationship between the biomarkers considered and the intake variables and the potential interaction of these dietary exposures with other nutritional, environmental and genetic factors constitutes a major challenge for interpretation. For this reason, and using structural equations modeling, estimation of measurement error in FFQ derived nutrients took into consideration two approaches, by including an alloyed gold standard with assumed hyperbolic relationship with biomarkers in plasma phospholipids (a biochemical approach) and two instrumental biomarkers with assumed linear relationship with intake (an epidemiological approach). Finally, although there has been evidence of correlation between intake of fatty acids and their levels in the substrates considered in our study, such a correlation does not necessarily render these biomarkers an adequate reflection of long-term fatty acid intake. In fact, the only substrate that has been shown to work as a gold standard is adipose tissue. However, because of the elevated cost and invasiveness of the procedure, studies using adipose tissue fatty acid concentration as an intake biomarker were often of limited sample size and hence correlations obtained had insufficient levels of precision (377, 379). Another potentially adequate biomarker that was often used to validate medium-term intake of fatty acids is erythrocyte membrane concentration (369, 378).

For my second and third papers, limitations included the lack of psychometric diagnosis for mild cognitive impairment, which might have been a more definite and clinically relevant outcome. However, the neuropsychological tests used represent some of the domains reported to be most sensitive in discriminating between normal aging and mild cognitive impairment (110).

The third paper had its own specific limitations which it shared with the second paper to a certain extent. First, it made use of tissue composition of fatty acids which does not necessarily reflect the proportion of fatty acids in the diet. Nevertheless, ARIC had also collected dietary intake data using a semi-quantitative food frequency questionnaire at the same baseline visit (i.e. visit 1). A study by Ma and colleagues had shown that intake of DHA and EPA was highly correlated with their concentrations in both cholesteryl esters and phospholipids (371). In addition, repeated measures of a sub-sample of plasma specimens indicated that fatty acid concentration in both fractions is reliable over the short and long term (423). Previous studies have also indicated that plasma levels of highly unsaturated fatty acids may also be good reflection of long-term dietary intake (372-375, 463). However, the main advantage of using a biomarker rather than a self-report of intake is that we are certain that errors in the exposure are independent of errors in our outcome measure. This would in most cases lead to an attenuation of effect in the presence of measurement error and hence lead to an odds ratio that is biased towards the null value of 1. Another limitation is that the sum of fatty acids in each fraction is 100. Therefore, a higher percentage of a specific group (e.g. SFA) will automatically reflect a lower percentage of another. Hence, there is a problem of interdependence which makes it difficult to interpret the effect of a single constituent or group of constituents. We are also unable to translate these findings quantitatively into dietary recommendations (e.g. number of servings of fish per week). Finally, a prior analysis indicated that there was a marked differential in short-term reliability between fatty acids that were major constituents (reliability coefficient > 0.65) and those that had a percentage less than 1% of total fatty acids(423).

D. Future Directions

Based on the first paper, future endeavors to correct for error should make use of structural equations modeling and include as many instrumental biomarkers as is available along with other self-reported or biomarker-based reference methods of dietary assessment. However, the choice of biomarkers and interpretation of their variability must be made as to account for biochemical and physiological interactions between dietary, environmental and genetic factors. Moreover, one must be cautious of coupled errors between biological markers and must take into account these correlations when specifying the structural model. Finally, because structural equations modeling makes a strong assumption about joint multivariate normality, often not present, it is crucial for future studies to use newly developed methodologies which appear to be more flexible in many ways (456).

According to my second paper, the literature indicates that these fatty acids were frequently found associated with reduced risk of cardiovascular disease, including stroke (327) and coronary heart disease (324, 325), although thus far all the evidence is of an observational nature. They have also been associated with improved insulin sensitivity(341), reduced risk of dyslipidemia (346) and a hypocoagulable profile (332) among other health benefits. Because many of these conditions are also related to cognitive impairment, future research should focus on disentangling the direct and indirect effects of fatty acids (using plasma biomarkers) on cognition and uncover the main mechanism involved in their ability to prevent clinically significant decline in aging populations. Finally, these findings suggest the utility of randomized clinical trials that would augment intake of marine fatty acids in the treatment group and give a non-enriched diet to the placebo group while allowing for stratification by baseline hypertensive status.

Based on findings from the third paper, there is reason to believe that subjects who are under increased oxidative stress, particularly hypertensive and dyslipidemic subjects, may have an added benefit to enriching their diet with ω-3 HUFAs which are mostly found in cold water fish (e.g. salmon, tuna, mackerel) and other foods of marine origin. Their increased intake may delay the progression of cognitive decline particularly in the area of verbal fluency. Future research should attempt to conduct subgroup analysis using actual markers of oxidative stress which have been developed recently (468). In addition, a stratified and randomized trial may be warranted comparing subjects with varying levels of oxidative stress in terms of their response to increasing dietary intake of ω-3 HUFAs using a more comprehensive battery of neuropsychological tests.

APPENDICES

APPENDIX A: Additional Methods and Results

$$pfh_6 = \left(1+\frac{C_6}{T_{6p}}\left(1+\frac{T_{3p}}{C_3}+\frac{T_o}{C_o}+\frac{T_{6p}}{K_S}\right)\right)^{-1} 100 \qquad \textbf{(A.1.)}$$

$$pfh_3 = \left(1+\frac{C_3}{T_{3p}}\left(1+\frac{T_{6p}}{C_6}+\frac{T_o}{C_o}+\frac{T_{3p}}{K_S}\right)\right)^{-1} 100 \qquad \textbf{(A.2.)}$$

$$pfh_6 = \left(1+\frac{PC_6}{T_{6p}}\left(1+\frac{T_{3p}}{PC_3}+\frac{T_{3p}}{HI_3}+\frac{T_o}{C_o}+\frac{T_{6p}}{K_S}\right)\right)^{-1} 100 + \left(1+\frac{HC_6}{T_{6H}}\left(1+\frac{T_{3H}}{HC_3}\right)\right)^{-1} 100$$

(A.3.)

TABLE A.1. Notations, Definitions and values: Estimating the alloyed gold standard T*

Notation	Definition	Value
C_3	Standard effective concentration of 18:3 (ω-3) as a percentage of total caloric intake	0.0400
C_6	Standard effective concentration of 18:2 (ω-6) as a percentage of total caloric intake	0.0600
C_o	Constant for the effect of other dietary fatty acids (non-essential).	5.0
K_S	Constant for shape fitting	0.175
PC_3	Standard effective concentration of 18:3 (ω-3) as a percentage of total caloric intake	0.0555
PC_6	Standard effective concentration of 18:2 (ω-6) as a percentage of total caloric intake	0.0441
HI_3	Competitive inhibition by the dietary ω-3 HUFA in elongation and desaturation of the (ω-3) and (ω-6) UFA.	0.005
HC_3	Efficiency of direct esterification of dietary (ω-3) HUFA.	3.0
HC_6	Efficiency of direct esterification of dietary (ω-6) HUFA.	0.70
HUFAs	20:3 ω-9, 20:3ω-6, 20:4 ω-6, 22:4 ω-6, 22:5 ω-6, 20:5 ω-3, 22:5 ω-3, 22:6 ω-3	
Pfh3	20:5+22:5ω-3 plasma phospholipids fatty acids as % of all HUFAs in the phospholipid fraction of plasma	
Pfh6	20:3+20:4 ω -6 plasma phospholipids fatty acids as % of all HUFAs in phospholipid fraction of plasma	
T_{3p}	% energy intake of: 18:3+18:4 ω-3	
T_{6p}	% energy intake of: 18:2+18:3 ω-6	
T_{3H}	% energy intake of: 20:5+22:5+22:6 ω-3	
T_{6H}	% energy intake of: 20:3+20:4+22:4+22:5 ω-6	
T_3	$T_{3p} + T_{3H}$	
T_6	$T_{6p} + T_{6H}$	
T_{3+6}	$T_3 + T_6$	
$T_{3H/6H}$	Ratio of T_{3H} to T_{6H}	
$T_{3P/6P}$	Ratio of T_{3P} to T_{6P}	
$T_{3/6}$	Ratio of (T_{3P}+T_{3H}) to (T_{6P}+T_{6H})	

A series of programming loop commands in STATA were used to first estimate the values of T_{3p}, T_{6p} and T_o using equations A..1 and A.2 and then to estimate T_{3H} and T_{6H}, given the now known values of the three other parameters, using equation A.3. An iterative process was used whereby permutation of values within a biologically plausible range was conducted and the appropriate combination was selected based on the minimum squared residual value of the biomarkers pfh_3 and pfh_6 and hence an attempt was made to improve the fit of the empirical equations model given observed values of biomarkers. The programming loop gave in each case one identifiable minimum. The biologically plausible values of T_{3p} were: 0.10-1.50 in increments of 0.01 percent; $T_o = Z \times T_{fmax}$ where T_{fmax}=50 and Z ranged from 0.10-1.00 in increments of 0.01; T_{6p} was constrained to range from 0.00 to 15.00 percent. Finally, using the third equation, T_{3H} was constrained to range from 0.01-1.00 percent in increments of 0.01 percent and T_{6H} was restricted to the range 0.01-0.50 percent in increments of 0.01 percent. The solution for $\{Z, T_{3p}, T_{6p}\}$ was obtained at a first stage, using the first two equations. These values were incorporated at a second stage into the third equation to obtain values for $\{T_{3H}, T_{6H}\}$. Similarly, the squared residual of pfh_6 , given equation A.3, was minimized to obtain the appropriate combination.

TABLE A.2. Regression calibration results using estimate of λ_j with its SE for various naïve estimates of β (SE=0.05)

	$\beta_{(naïve\ ,\ j)}$; SE=0.05									
	0.2000		0.4000		0.6000		0.8000		1.0000	
$\beta_{(RC,\ j)}$; (SE)										
6P	0.810	(0.062)	1.619	(0.032)	2.429	(0.021)	3.239	(0.016)	4.049	(0.012)
3P	1.143	(0.044)	2.286	(0.022)	3.429	(0.015)	4.571	(0.011)	5.714	(0.009)
6H	3.571	(0.014)	7.143	(0.007)	10.714	(0.005)	14.286	(0.004)	17.857	(0.003)
3H	0.490	(0.103)	0.980	(0.067)	1.471	(0.039)	1.961	(0.027)	2.451	(0.021)
6	0.952	(0.053)	1.905	(0.027)	2.857	(0.018)	3.810	(0.013)	4.762	(0.011)
3	0.597	(0.084)	1.194	(0.048)	1.791	(0.029)	2.388	(0.021)	2.985	(0.017)
3+6	0.909	(0.055)	1.818	(0.028)	2.727	(0.018)	3.636	(0.014)	4.545	(0.011)
3P/6P	0.513	(0.100)	1.026	(0.062)	1.538	(0.036)	2.051	(0.025)	2.564	(0.020)
3H/6H	0.405	(0.133)	0.810	(0.102)	1.215	(0.058)	1.619	(0.036)	2.024	(0.026)
3/6	0.512	(0.099)	1.023	(0.062)	1.535	(0.036)	2.046	(0.025)	2.558	(0.020)

We considered a structural model, in which *i* stands for individual and *j* for dietary variable *j* and T_{ij} (a latent variable) is the true value of dietary intake of nutrient or ratio of nutrients *j* for subject *i*. For that subject *i*, Q_{ij} is the value of dietary variable *j* derived from the food frequency questionnaire. T^*_{ij} is its value derived from an alloyed gold standard which was estimated from biomarkers in phospholipids fatty acid levels and biochemical empirical equations using STATA forval loops (381-383, 411). M_{ij} is the value of instrumental biomarker for nutrient or nutrient ratio *j* (plasma cholesteryl ester level of fatty acids). In addition, N_{ij} is another biomarker (plasma phospholipids level of fatty acids). Both of these biomarkers were shown previously to be highly correlated with reference methods of dietary assessment (374, 375, 438):

$$Q_{ij} = \alpha_{0Q_j} + \alpha_{1Q_j} T_{ij} + \varepsilon_{Q_{ij}}$$

$$T^*_{ij} = \alpha_{0T^*_j} + \alpha_{1T^*_j} T_{ij} + \varepsilon_{T^*_{ij}} \qquad \textbf{(A.4.)}$$

$$M_{ij} = \alpha_{0M_j} + \alpha_{1M_j} T_{ij} + \varepsilon_{M_{ij}}$$

$$N_{ij} = \alpha_{0N_j} + \alpha_{1N_j} T_{ij} + \varepsilon_{N_{ij}}$$

After imposing specific restrictions on this model, it was identified with varying degrees of freedom depending on variable *j* and an estimate of true intake for each dietary variable was obtained as a weighted average of measured variables in their standardized form. Subsequently, error in measurement from food frequency questionnaire was computed for each observation as: $\hat{u}_{ij} = Q_{ij} - \hat{T}_{ij}$. Hence, the error variance- covariance matrix could be derived accordingly. Our results were as follows, for the errors for variables *j* presented in the order {6P; 3P; 6H; 3H; 6; 3; 3+6; 3P/6P; 3H/6H; 3/6}:

TABLE A.3. Variance-covariance matrix of measurement error ($\hat{\Sigma}_{uu}$) in measured variables Q_j; ARIC (1987-89)

$\hat{\Sigma}_{uu}$ Variable *j*	6P	3P	6H	3H	6	3	3P/6P	3H/6H	3/6
6P	(1.506) *								
3P	0.207†	(1.054)							
6H	-0.356	0.021	(1.842)						
3H	-0.122	0.007	0.521	(1.222)					
6	1.286	0.131	0.038	-0.097	(1.315)				
3	-0.037	0.535	0.511	1.091	-0.025	(1.305)			
3+6	1.439	0.245	0.047	0.129	-0.480	0.370			
3P/6P	-0.421	0.514	-0.138	0.066	1.369	0.184	(0.906)		
3H/6H	0.047	-0.034	-0.129	0.911	-0.056	0.775	0.101	(1.021)	
3/6	-0.609	0.251	0.280	0.837	-0.599	0.932	0.713	0.608	(1.207)

* Numbers in parentheses constitute the variance of error for variable *j*.
† Off-diagonals are the covariances between measurement errors of variables *j*.

Although these estimates can be transported to other studies, we made use of the available data directly to estimate $\hat{\Sigma}_{uu}$ in each model, by considering standardized T*, M and N as replicates for standardized Q available for part of the data that can be used to estimate true value T. It is recommended to use the above matrix with SIMEX rather than RCAL which gives a bootstrap estimate of standard error for the measure of effect that is more consistent with our current method of application. For both cases (whether using replicate measures directly from the data or inputting the matrix by hand), the method of moments is used to correct for measurement error in covariates and can be summarized as follows: (445, 448):

$$\begin{pmatrix} \hat{\beta}_{Z,RC} \\ \hat{\beta}_{T,RC} \end{pmatrix} = \begin{pmatrix} \Sigma_{ZZ} & \Sigma_{ZQ} \\ \Sigma_{QZ} & \Sigma_{QQ} - \hat{\Sigma}_{uu} \end{pmatrix}^{-1} \begin{pmatrix} \Sigma_{ZZ} & \Sigma_{ZQ} \\ \Sigma_{QZ} & \Sigma_{QQ} \end{pmatrix} \begin{pmatrix} \hat{\beta}_{Z,naive} \\ \hat{\beta}_{T,naive} \end{pmatrix} \qquad \textbf{(A.5.)}$$

The generalized linear model in which measurement error correction of covariates is conducted can be written as:

$$\mathrm{E}(Y|Q_j, Z_k) = \hat{\beta}_0 + \sum_{j=1}^{m} \hat{\beta}_{\mathrm{T,naïve},j} Q_j + \sum_{k=1}^{n} \hat{\beta}_{\mathrm{Z,naïve},k} Z_k \qquad \textbf{(A.6.)}$$

Simulation extrapolation or SIMEX is a procedure consisting of four main steps:

(i) Fitting the causal model to obtain the estimated coefficients $\beta_{naïve}$ and an estimate of the measurement error variance σ^2_u. **(ii)** Generating random pseudo errors for a scale factor θ times the estimated error variance $\varepsilon \sim N(0, \theta\sigma^2_u)$.

These pseudo errors are added to the original values of the error prone covariate. Fit the model to obtain $\beta\{naïve, \theta_j\}$. This is repeated r times to obtain mean coefficient vector $\beta\{ \theta_j\}= (1/r)\Sigma \beta\{i, \theta_j\}$. **(iii)** The previous step is repeated for j=1 …, k* scale factors, where typically we use θ = {.5, 1, 1.5, 2}, though individual researchers may choose a longer list of scale factors. Using the typical list of scale factors, we have k=5 estimated coefficient vectors since k*=4 for the list above, and we have the estimated coefficient vector form the initial step (k=k*+1). **(iv)** For each regression coefficient β_m (m=1, …,p) in the model, we consider the estimated coefficient as a function of the scale factor θ_j for j=1, …k. Formally, we specify a function f( ) such that $\beta_m= f(\theta,\beta_m^{\{\theta\}})$. We estimate this relationship and then extrapolate back the final estimates $\beta_m= f(\theta_0= -1, \beta_m^{\{\theta\}})$ (no measurement error). Researchers are free to choose the form of the function f( ), but we point out that there are relatively few – in this case 5 – observations available to estimate the parameters of f( ). The function f( ) used to model the relationship between the estimated coefficient and θ is called the *extrapolant function* (446). Although deciding which model to fit is a valid question when performing SIMEX, it has been shown that conservative estimates with a quadratic curve do improve over the naïve estimator without any correction. Investigators may also use model fitting techniques to decide which model to fit and then extrapolate with. Calculating the standard error of the SIMEX estimator requires 100 simulations on its own. With the ever increasing speed of computers, the necessary computing power is widely available (447).

APPENDIX B: Instruments

DELAYED WORD RECALL TEST (DWRT)

O.M.B. 0925-0821
exp. 10/31/95

ARIC
Atherosclerosis Risk in Communities

COGNITIVE FUNCTION FORM

ID NUMBER: | | | | | | | | CONTACT YEAR: | 0 | 7 | FORM CODE: | C | N | F | VERSION: B 09/15/92

LAST NAME: | | | | | | | | | | | | | INITIALS: | | |

PART A: DELAYED WORD RECALL

PLACE A CHECK IN THE COLUMN TO THE RIGHT OF EACH WORD AFTER THE PARTICIPANT HAS READ IT ALOUD AND USED IT IN A SENTENCE.

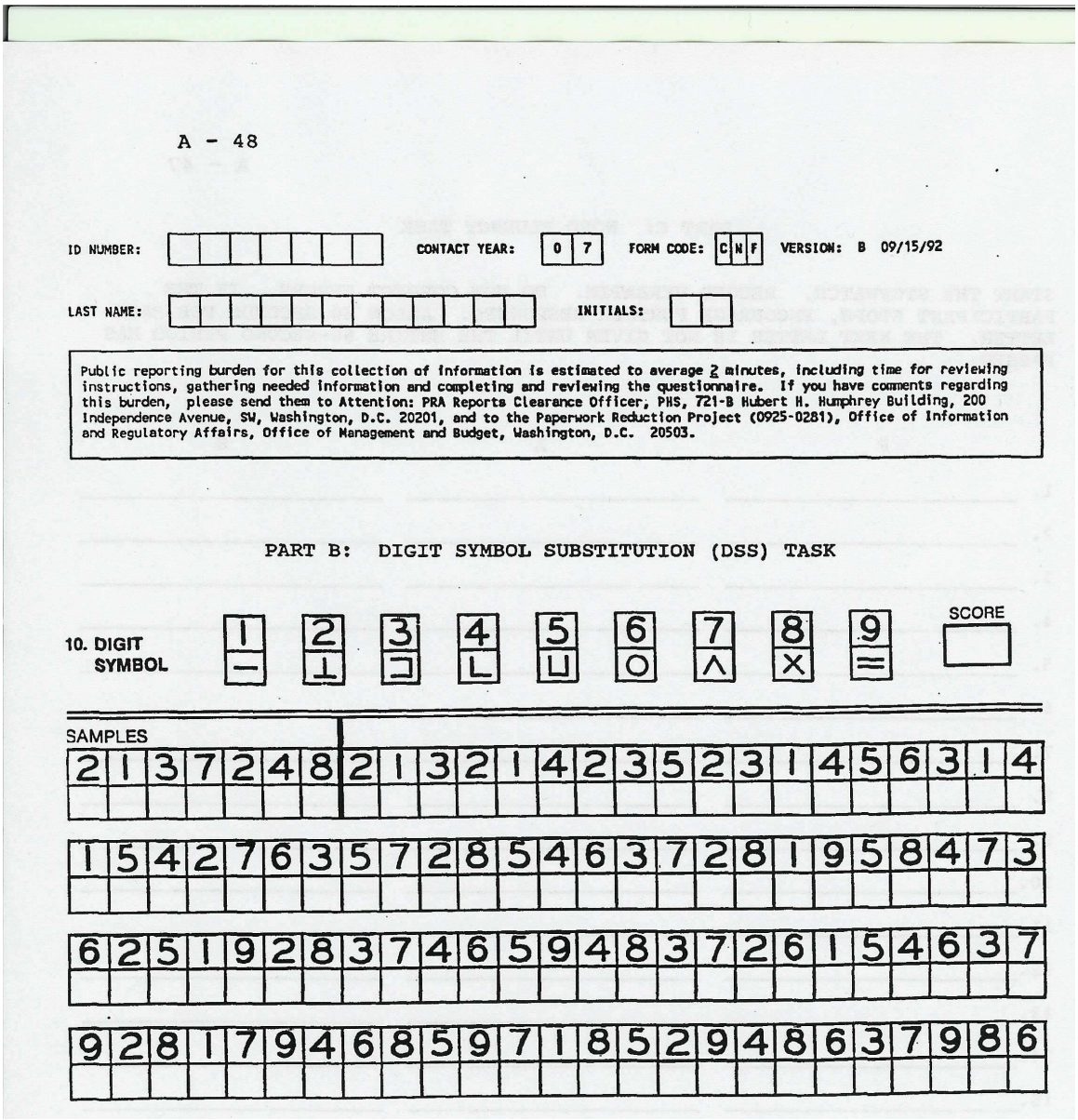
PLACE A CHECK IN THE 2ND COLUMN TO THE RIGHT OF EACH WORD AFTER THE PARTICIPANT HAS READ IT ALOUD AND USED IT IN A SENTENCE THE SECOND TIME.

AFTER THE COMPLETION OF THE DIGIT SYMBOL TEST, ASK THE PARTICIPANT TO RECALL THE 10 WORDS ORIGINALLY GIVEN:

CHECK OFF ALL THE WORDS RECALLED WITHIN 60 SECONDS.

	FIRST TIME	SECOND TIME	DELAYED WORD RECALL	
chimney	_____	_____	book	_____
salt	_____	_____	button	_____
harp	_____	_____	chimney	_____
button	_____	_____	finger	_____
meadow	_____	_____	flower	_____
train	_____	_____	harp	_____
flower	_____	_____	meadow	_____
finger	_____	_____	rug	_____
rug	_____	_____	salt	_____
book	_____	_____	train	_____

DIGIT SYMBOL SUBSTITUTION TEST (DSST/WAIS-R)



A - 48

ID NUMBER: CONTACT YEAR: 0 7 FORM CODE: C N F VERSION: B 09/15/92

LAST NAME: INITIALS:

Public reporting burden for this collection of information is estimated to average 2 minutes, including time for reviewing instructions, gathering needed information and completing and reviewing the questionnaire. If you have comments regarding this burden, please send them to Attention: PRA Reports Clearance Officer, PHS, 721-B Hubert H. Humphrey Building, 200 Independence Avenue, SW, Washington, D.C. 20201, and to the Paperwork Reduction Project (0925-0281), Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, D.C. 20503.

PART B: DIGIT SYMBOL SUBSTITUTION (DSS) TASK

10. DIGIT SYMBOL

1	2	3	4	5	6	7	8	9
—	⊥	⊐	L	⊔	O	^	X	=

SCORE

SAMPLES

2	1	3	7	2	4	8	2	1	3	2	1	4	2	3	5	2	3	1	4	5	6	3	1	4
1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3
6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7
9	2	8	1	7	9	4	6	8	5	9	7	1	8	5	2	9	4	8	6	3	7	9	8	6

WORD FLUENCY TEST (WFT)

PART C: WORD FLUENCY TASK

START THE STOPWATCH. RECORD VERBATIM. DO NOT CORRECT ERRORS. IF THE PARTICIPANT STOPS, ENCOURAGE FURTHER RESPONSES. ALLOW 60 SECONDS FOR EACH LETTER. THE NEXT LETTER IS NOT GIVEN UNTIL THE ENTIRE 60-SECOND PERIOD HAS PASSED.

	F	A	S
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			

COGNITIVE TEST SCORING SUMMARY

A - 46

CNF SCORING SUMMARY

PART A: DELAYED WORD RECALL

ADD UP THE CHECK MARKS IN COLUMN 3, PART A AND ENTER THE TOTAL NUMBER OF RECALLED WORDS BELOW:

1. TOTAL WORDS RECALLED (CNFB, Part A): | | |

PART B: DIGIT SYMBOL SUBSTITUTION

APPLY THE DSS SCORING TEMPLATE TO THE RESPONSES ON PART B AND ENTER THE NUMBER OF CORRECT SYMBOLS BELOW:

2. TOTAL CORRECT SYMBOLS (CNFB, Part B): | | |

APPLY THE DSS SCORING TEMPLATE TO THE RESPONSES ON PART B AND ENTER THE NUMBER OF INCORRECT SYMBOLS BELOW:

3. TOTAL INCORRECT SYMBOLS (CNFB, Part B): | | |

PART C: WORD FLUENCY

ADD UP THE TOTAL NUMBER OF WORDS LISTED IN COLUMNS F, A, AND S ON PART C, AND ENTER THAT TOTAL BELOW:

4. TOTAL WORDS LISTED (CNFB, Part C): | | |

PART D: ADMINISTRATIVE INFORMATION

5. DATE OF DATA COLLECTION: | | | / | | | / | | |
month day year

6. INTERVIEWER CODE NUMBER: | | | |

ARIC FOOD FREQUENCY QUESTIONNAIRE:

O.M.B. 0925-0281
exp. 7-31-89 A-184

ARIC DIETARY INTAKE FORM

ID NUMBER: | | | | | | | | CONTACT YEAR: | 0 | 1 | FORM CODE: | D | I | I | VERSION: A 11/1/86

LAST NAME: | | | | | | | | | | | | | | INITIALS: | | |

INSTRUCTIONS:
This form should be completed during the interview portion of the participant's visit. ID Number and Name must be entered above. Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost box. Enter leading zeroes where necessary to fill all boxes. If a number is entered incorrectly, mark through the incorrect entry with an "X". Code the correct entry clearly above the incorrect entry. For "multiple choice" and "yes/no" type questions, circle or write in the letter corresponding to the most appropriate response. If a letter is circled incorrectly, mark through it with an "X" and circle the correct response.

DIETARY INTAKE FORM (screen 1 of 18)

"In this part of the clinic visit we want to obtain information on your usual eating habits. We will go over specific foods by groups. I'll name a food and a portion size and you tell me how often, on average, you ate that during the past year.

If your portion was much different from the amount I say, please tell me if it was at least twice as much, or half as much. We have a few sizes of cups and glasses here for reference. Here are the choices for "how often" (show RC 1). The choices are number of times a day or week or month. Please respond with the appropriate letter. For example, "once a day" would be "D". If you ate or drank something less than twelve times a year, that would be the same as "less than once a month," which is "I".

It is important that your reply be brief in order to save time, but we want you to be as accurate as possible. If we miss food items that you usually eat often, we will list those at the end. Feel free to ask questions or have me repeat instructions if I am not being clear."

DIETARY INTAKE FORM (screen 2 of 18)

Response Categories:			
	>6 per day (A)	1 per day (D)	1 per week (G)
	4-6 per day (B)	5-6 per week (E)	1-3 per month (H)
	2-3 per day (C)	2-4 per week (F)	Almost Never (I)

A. [RC 1] DAIRY FOODS

"In the past year, how often on average did you consume..."

1. Skim or low fat milk; 8 oz. glass ..... ☐
2. Whole milk; 8 oz. glass ..... ☐
3. Yogurt; 1 c. ..... ☐
4. Ice cream; 1/2 c. ..... ☐
5. Cottage cheese or ricotta cheese; 1/2 c. ..... ☐
6. Other cheeses, plain or as part of a dish; 1 slice or serving..... ☐
7. Margarine or a margarine/butter blend; pats added to food or bread ..... ☐
8. Butter; pats added to food or bread ..... ☐

DIETARY INTAKE FORM (screen 3 of 18)

Response Categories:	>6 per day (A)	1 per day (D)	1 per week (G)
	4-6 per day (B)	5-6 per week (E)	1-3 per month (H)
	2-3 per day (C)	2-4 per week (F)	Almost Never (I)

B. [RC 1] FRUITS

"In the past year, how often on average did you consume..."

9. Fresh apples or pears; 1 .....
10. Oranges; 1 .....
11. Orange or grapefruit juice; small glass .....
12. Peaches, apricots or plums; 1 fresh or 1/2 c. canned or dried .....
13. Bananas; 1 .....
14. Other fruits; 1 fresh or 1/2 c. canned, including fruit cocktail .....

C. [RC 1] VEGETABLES -- Portion is 1/2 c.

"In the past year, how often on average did you consume..."

15. String beans or green beans; 1/2 c. .....
16. Broccoli; 1/2 c. .....

DIETARY INTAKE FORM (screen 4 of 18)

Response Categories:	>6 per day (A)	1 per day (D)	1 per week (G)
	4-6 per day (B)	5-6 per week (E)	1-3 per month (H)
	2-3 per day (C)	2-4 per week (F)	Almost Never (I)

17. Cabbage, cauliflower, brussels sprouts; 1/2 c.
18. Carrots; 1 whole or 1/2 c. cooked .....
19. Corn; 1 ear or 1/2 c. .....
20. Spinach, collards or other greens, but do not include lettuce; 1/2 c. .....
21. Peas or lima beans; 1/2 c. fresh, frozen or canned .....
22. Dark yellow, winter, squash such as acorn, butternut; 1/2 c. .....
23. Sweet potatoes; 1/2 c. .....
24. Beans or lentils, dried cooked, or canned, such as pinto, blackeye, baked beans; 1/2 c. .....
25. Tomatoes; 1, or tomato juice; 4 oz. .....

DIETARY INTAKE FORM (screen 5 of 18)

Response Categories:	>6 per day (A)	1 per day (D)	1 per week (G)
	4-6 per day (B)	5-6 per week (E)	1-3 per month (H)
	2-3 per day (C)	2-4 per week (F)	Almost Never (I)

D. [RC 1] MEATS

"In the past year, how often on average did you consume..."

26. Chicken or turkey, without skin .....
27. Chicken or turkey, with skin .....
28. Hamburgers; 1 .....
29. Hot dogs; 1 .....
30. Processed meats: sausage, salami, bologna, etc.; piece or slice .....
31. Bacon; 2 slices .....
32. Beef, pork or lamb as a sandwich or mixed dish, stew, casserole, lasagne, or in spaghetti sauce, etc. .....
33. Beef, pork or lamb as a main dish, steak, roast, ham, etc.....
34. Canned tuna fish; 3-4 oz. .....

DIETARY INTAKE FORM (screen 6 of 18)

Response Categories:	>6 per day (A)	1 per day (D)	1 per week (G)
	4-6 per day (B)	5-6 per week (E)	1-3 per month (H)
	2-3 per day (C)	2-4 per week (F)	Almost Never (I)

35. Dark meat fish, such as salmon, mackerel, swordfish, sardines, bluefish; 3-5 oz. .....
36. Other fish, such as cod, perch, catfish, etc.; 3-5 oz. .....
37. Shrimp, lobster, scallops as a main dish .....
38. Eggs; 1 .....

E. [RC 1] SWEETS, BAKED GOODS, CEREALS

"In the past year, how often on average did you consume..."

39. Chocolate bars or pieces, such as Hershey's, Plain M & M's, Snickers, Reeses; 1 oz. .....
40. Candy without chocolate; 1 oz.....
41. Pie, homemade from scratch; 1 slice .....

DIETARY INTAKE FORM (screen 7 of 18)

Response Categories:	>6 per day (A)	1 per day (D)	1 per week (G)
	4-6 per day (B)	5-6 per week (E)	1-3 per month (H)
	2-3 per day (C)	2-4 per week (F)	Almost Never (I)

42. Pie, ready-made or from a mix; 1 slice .....
43. Donut; 1 .....
44. Biscuits or cornbread; 1 .....
45. Danish pastry, sweet roll, coffee cake, croissant; 1 .....
46. Cake or brownie; 1 piece .....
47. Cookies; 1 .....
48. Cold breakfast cereal; 1/2 c. .....
49. Cooked cereals such as oatmeal, grits, cream of wheat; 1/2 c.....
50. White bread; 1 slice .....
51. Dark or whole grain bread; 1 slice .....

F. [RC 1] MISCELLANEOUS

"In the past year, how often on average did you consume..."

52. Peanut butter; 1 tbsp .....

DIETARY INTAKE FORM (screen 8 of 18)

Response Categories:	>6 per day (A)	1 per day (D)	1 per week (G)
	4-6 per day (B)	5-6 per week (E)	1-3 per month (H)
	2-3 per day (C)	2-4 per week (F)	Almost Never (I)

53. Potato chips or corn chips; small bag or 1 oz.
54. French fried potatoes; 1 serving, 4 oz. .....
55. Nuts; 1 oz. .....
56. Potatoes, mashed; 1 c. or baked; 1 .....
57. Rice; 1/2 c. .....
58. Spaghetti, noodles or other pasta; 1/2 c. ....
59. Home-fried food, such as any meats, poultry, fish, shrimp, eggs, vegetables, etc.; 1 serving .....
60. Food fried away from home, such as any fish, chicken, chicken nuggets, etc. .....

DIETARY INTAKE FORM (screen 9 of 18)

Response Categories:			
	>6 per day (A)	1 per day (D)	1 per week (G)
	4-6 per day (B)	5-6 per week (E)	1-3 per month (H)
	2-3 per day (C)	2-4 per week (F)	Almost Never (I)

G. [RC 1] BEVERAGES

"In the past year, how often on average did you consume..."

61. Coffee, not decaffeinated; 1 c. ..... ☐

62. Tea, iced or hot, not including decaf or herbal tea; 1 cup ..... ☐

63. Low calorie soft drinks, such as any diet Coke, diet Pepsi, diet 7-Up; 1 glass ..... ☐

64. Regular soft drinks, such as Coke, Pepsi, 7-Up, ginger ale; 1 glass ..... ☐

65. Fruit-flavored punch or non-carbonated beverages, such as lemonade, Kool-Aid or Hawaiian Punch; not diet; 1 glass ..... ☐

DIETARY INTAKE FORM (screen 10 of 18)

H. OTHER DIETARY ITEMS

66. [RC 2] How often do you eat liver; 3-4 oz. serving? ..... 1/week A
2-3/month B
1/month or less C
Never D

67. Are there any other foods that you usually eat at least twice per week such as tortillas, prunes, or avocado? Do not include dry spices nor something that has been listed previously. ..... Yes Y
No N

Go to Item 74, Screen 11

68. Food #1 eaten at least twice per week (enter code and specify food and usual portion size below):.. ☐☐☐

a. _____

69. [RC 3] Frequency for food #1: ..... > 6/day A
4-6/day B
2-3/day C
1/day D
5-6/wk E
2-4/wk F

DIETARY INTAKE FORM (screen 11 of 18)

70. Food #2 eaten at least twice per week (enter code and specify food and usual portion size below):.. ☐☐☐

a. _____

71. [RC 3] Frequency for food #2: ..... > 6/day A
4-6/day B
2-3/day C
1/day D
5-6/wk E
2-4/wk F

72. Food #3 eaten at least twice per week (enter code and specify food and usual portion size below):.. ☐☐☐

a. _____

73. [RC 3] Frequency for food #3: ..... > 6/day A
4-6/day B
2-3/day C
1/day D
5-6/wk E
2-4/wk F

74. [RC 4] What do you do with the visible fat on your meat? .....
Eat most of the fat A
Eat some of the fat B
Eat as little as possible C
Don't eat meat D

DIETARY INTAKE FORM (screen 12 of 18)

75. [RC 5] What kind of fat do you usually use for frying and sauteing foods at home, excluding "Pam"-type spray? .....

Go to Item 77
- Real Butter A
- Margarine B
- Vegetable Oil C
- Vegetable Shortening D
- Lard E
- Bacon Grease F
- Not Applicable G
- Unknown H

76. Enter code and specify brand and form below: ..... ☐☐☐

a. _____

77. [RC 5] What kind of fat do you usually use for baking? .....

Go to Item 79, Screen 13
- Real Butter A
- Margarine B
- Vegetable Oil C
- Vegetable Shortening D
- Lard E
- Bacon Grease F
- Not Applicable G
- Unknown H

78. Enter code and specify brand and form below: ..... ☐☐☐

a. _____

DIETARY INTAKE FORM (screen 13 of 18)

79. [RC 6] What brand and form of margarine do you usually use at the table?

a. Form: ..... None A

Go to Item 80

Stick B

Tub C

Diet (low calorie) D

Other E

b. Code number: ..... ☐☐☐

c. Brand: _____

80. What kind of cold breakfast cereal do you most often use? (Enter code and specify brand name below): ..... ☐☐☐

a. Brand: _____

81. Are you currently on a special diet? ..... Yes Y

Go to Item 84, Screen 14

No N

82. For how many years have you been on it? .. ☐☐

83. [RC 7] What type of diet is it? ...

Weight Loss A

Low Salt B

Low Cholesterol C

Weight Gain D

Diabetic E

Other F

DIETARY INTAKE FORM (screen 14 of 18)

84. How many teaspoons of sugar do you add to your food daily? Include sugar added to coffee, tea, cereal, etc. ..... ☐☐

85. [RC 8] In cooking vegetables, how often do you add fat such as salt pork, butter, or margarine? .....

2-3 times per day A

1 time per day B

5-6 times per week C

2-4 times per week D

1 time per week E

1-3 times per month F

Never G

Unknown H

86. [RC 8] How often is salt or salt-containing seasoning such as garlic salt, onion salt, soy sauce, or Accent added to your food in cooking? .....

2-3 times per day A

1 time per day B

5-6 times per week C

2-4 times per week D

1 time per week E

1-3 times per month F

Never G

Unknown H

87. How many shakes of salt do you add to your food at the table every day? .... ☐☐

DIETARY INTAKE FORM (screen 15 of 18)

88. [RC 8] How often do you add catsup, hot sauce, soy or steak sauces to your food? ....

2-3 times per day	A
1 time per day	B
5-6 times per week	C
2-4 times per week	D
1 time per week	E
1-3 times per month	F
Never	G
Unknown	H

89. [RC 8] How often do you eat special low salt foods such as low salt chips, nuts, cheese, or salad dressing? .....

2-3 times per day	A
1 time per day	B
5-6 times per week	C
2-4 times per week	D
1 time per week	E
1-3 times per month	F
Never	G
Unknown	H

DIETARY INTAKE FORM (screen 16 of 18)

I. ALCOHOL

"I am going to ask you about wine, beer, and drinks made with hard liquor because these are the three major types of alcoholic beverages."

90. Do you presently drink alcoholic beverages? ..... Yes Y

No N

Go to Item 96, Screen 17

91. Have you ever consumed alcoholic beverages? ..... Yes Y

No N

Go to Item 101, Screen 18

92. Approximately how many years ago did you stop drinking? ..... ☐☐

93. For how many years did you drink alcoholic beverages? ..... ☐☐

94. In the past, which types of alcoholic beverages did you ordinarily drink? {Circle Y or N for each type below}

	Yes	No
a. Wine	Y	N
b. Beer	Y	N
c. Drinks made with hard liquor	Y	N
d. Other	Y	N
e. Specify: ☐☐☐☐☐☐☐☐☐☐		

DIETARY INTAKE FORM (screen 17 of 18)

95. What was the usual number of drinks you had per week before you stopped drinking alcoholic beverages? ..... ☐☐
{One drink means 1 beer or 1 glass of wine or 1 shot of liquor or 1 mixed drink. Record 0 if less than one drink per week.}

After completing item 95, go to item 101

96. How many glasses of wine do you usually have per week? ..... ☐☐
{4 oz. glasses; round down}

97. How many bottles or cans of beer do you usually have per week? ..... ☐☐
{12 oz. bottles or cans; round down}

98. How many drinks of hard liquor do you usually have per week? ..... ☐☐
{1 1/2 oz. shots; round down}

99. During the past 24 hours, how many drinks have you had? ..... ☐☐

If "0", go to item 101

100. Were these: {Circle Y or N for each}

	Yes	No
a. Wine?	Y	N
b. Beer?	Y	N
c. Liquor?	Y	N

DIETARY INTAKE FORM (screen 18 of 18)

J. WEIGHT AT AGE 25

101. What was your weight at age 25? (pounds) ..... ☐☐☐

K. ADMINISTRATIVE INFORMATION

102. Interviewer's opinion of information: ....

Reliable	A
Questionable	B
Participant uncooperative	C
Participant unable to estimate frequencies	D

103. Date of data collection: ... ☐☐ - ☐☐ - ☐☐
Month Day Year

104. Method of data collection: ..... Computer C
Paper Form P

105. Code number of person completing this form: ... ☐☐☐

BIBLIOGRAPHY

1. United Nations. World Population Prospects: The 2002 revision.; 2002.

2. LaCroix AZ, Guralnik JM, Berkman LF, Wallace RB, Satterfield S. Maintaining mobility in late life. II. Smoking, alcohol consumption, physical activity, and body mass index. Am J Epidemiol 1993;137(8):858-69.

3. Verbrugge LM, Jette AM. The disablement process. Soc Sci Med 1994;38(1):1-14.

4. Stuck AE, Walthert JM, Nikolaus T, Bula CJ, Hohmann C, Beck JC. Risk factors for functional status decline in community-living elderly people: a systematic literature review. Soc Sci Med 1999;48(4):445-69.

5. Moritz DJ, Kasl SV, Berkman LF. Cognitive functioning and the incidence of limitations in activities of daily living in an elderly community sample. Am J Epidemiol 1995;141(1):41-9.

6. Mehta KM, Yaffe K, Covinsky KE. Cognitive impairment, depressive symptoms, and functional decline in older people. J Am Geriatr Soc 2002;50(6):1045-50.

7. Sauvaget C, Yamada M, Fujiwara S, Sasaki H, Mimori Y. Dementia as a predictor of functional disability: a four-year follow-up study. Gerontology 2002;48(4):226-33.

8. Raji MA, Al Snih S, Ray LA, Patel KV, Markides KS. Cognitive status and incident disability in older Mexican Americans: findings from the Hispanic established population for the epidemiological study of the elderly. Ethn Dis 2004;14(1):26-31.

9. Arab L. Biomarkers of fat and fatty acid intake. J Nutr 2003;133 Suppl 3:925S-932S.

10. Youdim KA, Martin A, Joseph JA. Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci 2000;18(4-5):383-99.

11. Andreassi M, Forleo P, Di Lorio A, Masci S, Abate G, Amerio P. Efficacy of gamma-linolenic acid in the treatment of patients with atopic dermatitis. J Int Med Res 1997;25(5):266-74.

12. Cerolini S, Kelso KA, Noble RC, Speake BK, Pizzi F, Cavalchini LG. Relationship between spermatozoan lipid composition and fertility during aging of chickens. Biol Reprod 1997;57(5):976-80.

13. Zhang L. The effects of essential fatty acids preparation in the treatment of intrauterine growth retardation. Am J Perinatol 1997;14(9):535-7.

14. Bjerve KS. Omega 3 fatty acid deficiency in man: implications for the requirement of alpha-linolenic acid and long-chain omega 3 fatty acids. World Rev Nutr Diet 1991;66:133-42.

15. Wainwright PE. Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. Proc Nutr Soc 2002;61(1):61-9.

16. Tinoco J. Dietary requirements and functions of alpha-linolenic acid in animals. Prog Lipid Res 1982;21(1):1-45.

17.Lopez GH, Ilincheta de Boschero MG, Castagnet PI, Giusto NM. Age-associated changes in the content and fatty acid composition of brain glycerophospholipids. Comp Biochem Physiol B Biochem Mol Biol 1995;112(2):331-43.

18.Youdim KA, Deans SG. Beneficial effects of thyme oil on age-related changes in the phospholipid C20 and C22 polyunsaturated fatty acid composition of various rat tissues. Biochim Biophys Acta 1999;1438(1):140-6.

19.Haag M. Essential fatty acids and the brain. Can J Psychiatry 2003;48(3):195-203.

20.Champeil-Potokar G, Denis I, Goustard-Langelier B, Alessandri JM, Guesnet P, Lavialle M. Astrocytes in culture require docosahexaenoic acid to restore the n-3/n-6 polyunsaturated fatty acid balance in their membrane phospholipids. J Neurosci Res 2004;75(1):96-106.

21.Yamamoto N, Okaniwa Y, Mori S, Nomura M, Okuyama H. Effects of a high-linoleate and a high-alpha-linolenate diet on the learning ability of aged rats. Evidence against an autoxidation-related lipid peroxide theory of aging. J Gerontol 1991;46(1):B17-22.

22.Lamptey MS, Walker BL. A possible essential role for dietary linolenic acid in the development of the young rat. J Nutr 1976;106(1):86-93.

23.Yehuda S, Carasso RL. Modulation of learning, pain thresholds, and thermoregulation in the rat by preparations of free purified alpha-linolenic and linoleic acids: determination of the optimal omega 3-to-omega 6 ratio. Proc Natl Acad Sci U S A 1993;90(21):10345-9.

24.Enslen M, Milon H, Malnoe A. Effect of low intake of n-3 fatty acids during development on brain phospholipid fatty acid composition and exploratory behavior in rats. Lipids 1991;26(3):203-8.

25.Wainwright PE, Xing HC, Girard T, Parker L, Ward GR. Effects of dietary n-3 fatty acid deficiency on Morris water-maze performance and amphetamine-induced conditioned place preference in rat. Nutritional Neuroscience 1998;1:281-293.

26.Moriguchi T, Greiner RS, Salem N, Jr. Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. J Neurochem 2000;75(6):2563-73.

27.Yamamoto N, Saitoh M, Moriuchi A, Nomura M, Okuyama H. Effect of dietary alpha-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. J Lipid Res 1987;28(2):144-51.

28.Umezawa M, Ohta A, Tojo H, Yagi H, Hosokawa M, Takeda T. Dietary alpha-linolenate/linoleate balance influences learning and memory in the senescence-accelerated mouse (SAM). Brain Res 1995;669(2):225-33.

29.de Wilde MC, Hogyes E, Kiliaan AJ, Farkas T, Luiten PG, Farkas E. Dietary fatty acids alter blood pressure, behavior and brain membrane composition of hypertensive rats. Brain Res 2003;988(1-2):9-19.

30.Murphy MG. Membrane fatty acids, lipid peroxidation and adenylate cyclase activity in cultured neural cells. Biochem Biophys Res Commun 1985;132(2):757-63.

31.Nicolas C, Lacasa D, Giudicelli Y, Demarne Y, Agli B, Lecourtier MJ, et al. Dietary (n-6) polyunsaturated fatty acids affect beta-adrenergic receptor binding and adenylate cyclase activity in pig adipocyte plasma membrane. J Nutr 1991;121(8):1179-86.

32.Speizer LA, Watson MJ, Brunton LL. Differential effects of omega-3 fish oils on protein kinase activities in vitro. Am J Physiol 1991;261(1 Pt 1):E109-14.

33.Irvine RF, Letcher AJ, Dawson RM. Fatty acid stimulation of membrane phosphatidylinositol hydrolysis by brain phosphatidylinositol phosphodiesterase. Biochem J 1979;178(2):497-500.

34. McPhail LC, Clayton CC, Snyderman R. A potential second messenger role for unsaturated fatty acids: activation of Ca2+-dependent protein kinase. Science 1984;224(4649):622-5.

35. Horrobin DF, Bennett CN. Depression and bipolar disorder: relationships to impaired fatty acid and phospholipid metabolism and to diabetes, cardiovascular disease, immunological abnormalities, cancer, ageing and osteoporosis. Possible candidate genes. Prostaglandins Leukot Essent Fatty Acids 1999;60(4):217-34.

36. Kearns SD, Haag M. The effect of omega-3 fatty acids on Ca-ATPase in rat cerebral cortex. Prostaglandins Leukot Essent Fatty Acids 2002;67(5):303-8.

37.Yehuda S, Rabinovitz S, Mostofsky DI. Essential fatty acids are mediators of brain biochemistry and cognitive functions. J Neurosci Res 1999;56(6):565-70.

38.de Gomez Dumm IN, de Alaniz MJ, Brenner RR. Effect of dietary fatty acids on delta 5 desaturase activity and biosynthesis of arachidonic acid in rat liver microsomes. Lipids 1983;18(11):781-8.

39.Mahfouz MM, Smith TL, Kummerow FA. Effect of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat-liver microsomes. Lipids 1984;19(3):214-22.

40.Garg ML, Sebokova E, Thomson AB, Clandinin MT. Delta 6-desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or omega 3 fatty acids. Biochem J 1988;249(2):351-6.

41.Mason RP, Walter MF, Mason PE. Effect of oxidative stress on membrane structure: small-angle X-ray diffraction analysis. Free Radic Biol Med 1997;23(3):419-25.

42.Tirosh O, Kohen R, Katzhendler J, Alon A, Barenholz Y. Oxidative stress effect on the integrity of lipid bilayers is modulated by cholesterol level of bilayers. Chem Phys Lipids 1997;87(1):17-22.

43.Bourre JM, Dumont OS, Piciotti MJ, Pascal GA, Durand GA. Dietary alpha-linolenic acid deficiency in adult rats for 7 months does not alter brain docosahexaenoic acid content, in contrast to liver, heart and testes. Biochim Biophys Acta 1992;1124(2):119-22.

44.Alves de Moraes SA, Szklo M, Knopman D, Sato R. The relationship between temporal changes in blood pressure and changes in cognitive function: Atherosclerosis Risk in Communities (ARIC) Study. Preventive Medicine 2002;33:258-263.

45.Starr JM. Blood pressure and cognitive decline in the elderly. Curr Opin Nephrol Hypertens 1999;8(3):347-51.

46.Elias MF. Effects of chronic hypertension on cognitive functioning. Geriatrics 1998;53 Suppl 1:S49-52.

47.Elias MF, Elias PK, Sullivan LM, Wolf PA, D'Agostino RB. Lower cognitive function in the presence of obesity and hypertension: the Framingham heart study. Int J Obes Relat Metab Disord 2003;27(2):260-8.

48.Edmond J. Essential polyunsaturated fatty acids and the barrier to the brain: the components of a model for transport. J Mol Neurosci 2001;16(2-3):181-93; discussion 215-21.

49.Moore SA. Polyunsaturated fatty acid synthesis and release by brain-derived cells in vitro. J Mol Neurosci 2001;16(2-3):195-200; discussion 215-21.

50.Tang JP, Xu ZQ, Douglas FL, Rakhit A, Melethil S. Increased blood-brain barrier permeability of amino acids in chronic hypertension. Life Sci 1993;53(25):PL417-20.

51.Yamagata K, Tagami M, Nara Y, Fujino H, Kubota A, Numano F, et al. Faulty induction of blood-brain barrier functions by astrocytes isolated from stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 1997;24(9-10):686-91.

52.de Champlain J, Wu R, Girouard H, Karas M, A ELM, Laplante MA, et al. Oxidative stress in hypertension. Clin Exp Hypertens 2004;26(7-8):593-601.

53.Wesnes K. Assessing cognitive function in clinical trials: latest developments and future directions. Drug Discov Today 2002;7(1):29-35.

54.Lindeboom J, Weinstein H. Neuropsychology of cognitive ageing, minimal cognitive impairment, Alzheimer's disease, and vascular cognitive impairment. Eur J Pharmacol 2004;490(1-3):83-6.

55.Verhaeghen P, Salthouse TA. Meta-analyses of age-cognition relations in adulthood: estimates of linear and nonlinear age effects and structural models. Psychol Bull 1997;122(3):231-49.
56. Stern Y. The concept of cognitive reserve: a catalyst for research. J Clin Exp Neuropsychol 2003;25(5):589-93.

57.Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297(5580):353-6.

58.Turner RS. Biomarkers of Alzheimer's disease and mild cognitive impairment: are we there yet? Exp Neurol 2003;183(1):7-10.

59.Blennow K, Vanmechelen E, Hampel H. CSF total tau, Abeta42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. Mol Neurobiol 2001;24(1-3):87-97.

60.Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Neurobiol Aging 1997;18(4 Suppl):S1-2.

61.O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, et al. Vascular cognitive impairment. Lancet Neurol 2003;2(2):89-98.

62. Crecelius C. Diagnosis and treatment of non-Alzheimer's dementias. J Am Med Dir Assoc 2003;4(4 Suppl):H25-9.

63.McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34(7):939-44.

64.Roman GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993;43(2):250-60.

65. Hachinski VC, Iliff LD, Zilhka E, Du Boulay GH, McAllister VL, Marshall J, et al. Cerebral blood flow in dementia. Arch Neurol 1975;32(9):632-7.

66.Clinical and neuropathological criteria for frontotemporal dementia. The Lund and Manchester Groups. J Neurol Neurosurg Psychiatry 1994;57(4):416-8.

67. McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 1996;47(5):1113-24.

68. Zekry D, Hauw JJ, Gold G. Mixed dementia: epidemiology, diagnosis, and treatment. J Am Geriatr Soc 2002;50(8):1431-8.

69.Lim A, Tsuang D, Kukull W, Nochlin D, Leverenz J, McCormick W, et al. Clinico-neuropathological correlation of Alzheimer's disease in a community-based case series. J Am Geriatr Soc 1999;47(5):564-9.

70.Massoud F, Devi G, Stern Y, Lawton A, Goldman JE, Liu Y, et al. A clinicopathological comparison of community-based and clinic-based cohorts of patients with dementia. Arch Neurol 1999;56(11):1368-73.

71.Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Lancet 2001;357(9251):169-75.

72.Langa KM, Foster NL, Larson EB. Mixed dementia: emerging concepts and therapeutic implications. Jama 2004;292(23):2901-8.

73.World Health Organization. The ICD-10 Classification of Mental and Behavioral Disorders: Diagnostic Criteria for Research. Geneva, Switzerland: World Health Organization; 1993.
74.APA. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Washington, DC: American Psychiatric Association; 1994.

75.Chui HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. Neurology 1992;42(3 Pt 1):473-80.

76. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. J Neural Transm 2002;109(5-6):813-36.

77. Lobo A, Launer LJ, Fratiglioni L, Andersen K, Di Carlo A, Breteler MM, et al. Prevalence of dementia and major subtypes in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology 2000;54(11 Suppl 5):S4-9.

78. Li G, Shen YC, Chen CH, Zhao YW, Li SR, Lu M. An epidemiological survey of age-related dementia in an urban area of Beijing. Acta Psychiatr Scand 1989;79(6):557-63.

79. Fichter MM, Meller I, Schroppel H, Steinkirchner R. Dementia and cognitive impairment in the oldest old in the community. Prevalence and comorbidity. Br J Psychiatry 1995;166(5):621-9.

80. Ankri J, Poupard M. [Prevalence and incidence of dementia among the very old. Review of the literature]. Rev Epidemiol Sante Publique 2003;51(3):349-60.

81. Jorm AF, Jolley D. The incidence of dementia: a meta-analysis. Neurology 1998;51(3):728-33.

82. Ott A, Breteler MM, van Harskamp F, Claus JJ, van der Cammen TJ, Grobbee DE, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam study. Bmj 1995;310(6985):970-3.

83. Liu HC, Lin KN, Teng EL, Wang SJ, Fuh JL, Guo NW, et al. Prevalence and subtypes of dementia in Taiwan: a community survey of 5297 individuals. J Am Geriatr Soc 1995;43(2):144-9.

84. Simpson PM, et al. The cognitive drug research computerized assessment system for demented patients: a validation study. International Journal of Geriatric Psychiatry 1991;6:95-102.

85. Nebes RD, Brady CB. Focused and divided attention in Alzheimer's disease. Cortex 1989;25(2):305-15.

86. Wesnes K. The effects of psychotropic drugs upon human behaviour. Mod Probl Pharmacopsychiatry 1977;12:37-58.

87. Bowen J, Teri L, Kukull W, McCormick W, McCurry SM, Larson EB. Progression to dementia in patients with isolated memory loss. Lancet 1997;349(9054):763-5.

88. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999;56(3):303-8.

89. DeCarli C. Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. Lancet Neurol 2003;2(1):15-21.

90. Livingston G, Sax K, Willison J, Blizard B, Mann A. The Gospel Oak Study stage II: the diagnosis of dementia in the community. Psychol Med 1990;20(4):881-91.

91. Fratiglioni L, De Ronchi D, Aguero-Torres H. Worldwide prevalence and incidence of dementia. Drugs Aging 1999;15(5):365-75.

92. White L, Petrovitch H, Ross GW, Masaki KH, Abbott RD, Teng EL, et al. Prevalence of dementia in older Japanese-American men in Hawaii: The Honolulu-Asia Aging Study. Jama 1996;276(12):955-60.

93.Bachman DL, Wolf PA, Linn R, Knoefel JE, Cobb J, Belanger A, et al. Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. Neurology 1992;42(1):115-9.

94. Skoog I, Nilsson L, Palmertz B, Andreasson LA, Svanborg A. A population-based study of dementia in 85-year-olds. N Engl J Med 1993;328(3):153-8.

95.Aronson MK, Ooi WL, Geva DL, Masur D, Blau A, Frishman W. Dementia. Age-dependent incidence, prevalence, and mortality in the old old. Arch Intern Med 1991;151(5):989-92.

96.Shibayama H, Kasahara Y, Kobayashi H. Prevalence of dementia in a Japanese elderly population. Acta Psychiatr Scand 1986;74(2):144-51.

97.Hasegawa K. The clinical issues of age-related dementia. Tohoku J Exp Med 1990;161 Suppl:29-38.

98.Zhang MY, Katzman R, Salmon D, Jin H, Cai GJ, Wang ZY, et al. The prevalence of dementia and Alzheimer's disease in Shanghai, China: impact of age, gender, and education. Ann Neurol 1990;27(4):428-37.

99. Fitzpatrick AL, Kuller LH, Ives DG, Lopez OL, Jagust W, Breitner JC, et al. Incidence and prevalence of dementia in the Cardiovascular Health Study. J Am Geriatr Soc 2004;52(2):195-204.

100.Kral VA. Senescent forgetfulness: benign and malignant. Can Med Assoc J 1962;86:257-60.

101.Crook T, Bartus RT, Ferris SH, Whitehouse P, Cohen GD, Gershon S. Age-associated memory impairment: proposed diagnostic criteria and measures of clinical change -- Report of a National Institute of Mental Health work group. Developmental Neuropsychology 1986;2:261-276.

102.Blackford RC, LaRue A. Criteria for diagnosing age associated memory impairment: proposed improvements from the field. Developmental Neuropsychology 1989;5:295-306.

103.Ebly EM, Hogan DB, Parhad IM. Cognitive impairment in the nondemented elderly. Results from the Canadian Study of Health and Aging. Arch Neurol 1995;52(6):612-9.

104.Graham JE, Rockwood K, Beattie BL, Eastwood R, Gauthier S, Tuokko H, et al. Prevalence and severity of cognitive impairment with and without dementia in an elderly population. Lancet 1997;349(9068):1793-6.

105.Canadian study of health and aging: study methods and prevalence of dementia. Cmaj 1994;150(6):899-913.

106.Hogan DB, Ebly EM. Predicting who will develop dementia in a cohort of Canadian seniors. Can J Neurol Sci 2000;27(1):18-24.

107. Fisk JD, Merry HR, Rockwood K. Variations in case definition affect prevalence but not outcomes of mild cognitive impairment. Neurology 2003;61(9):1179-84.

108.Petersen RC, Smith GE, Ivnik RJ, Tangalos EG, Schaid DJ, Thibodeau SN, et al. Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. Jama 1995;273(16):1274-8.

109.Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, et al. Current concepts in mild cognitive impairment. Arch Neurol 2001;58(12):1985-92.

110.Ritchie K, Artero S, Touchon J. Classification criteria for mild cognitive impairment: a population-based validation study. Neurology 2001;56(1):37-42.

111.Koivisto K, Reinikainen KJ, Hanninen T, Vanhanen M, Helkala EL, Mykkanen L, et al. Prevalence of age-associated memory impairment in a randomly selected population from eastern Finland. Neurology 1995;45(4):741-7.

112.Barker A, Jones R, Jennison C. A prevalence study of age-associated memory impairment. Br J Psychiatry 1995;167(5):642-8.

113.Tabert MH, Albert SM, Borukhova-Milov L, Camacho Y, Pelton G, Liu X, et al. Functional deficits in patients with mild cognitive impairment: prediction of AD. Neurology 2002;58(5):758-64.

114.Jelic V, Johansson SE, Almkvist O, Shigeta M, Julin P, Nordberg A, et al. Quantitative electroencephalography in mild cognitive impairment: longitudinal changes and possible prediction of Alzheimer's disease. Neurobiol Aging 2000;21(4):533-40.

115.Jack CR, Jr., Petersen RC, Xu YC, O'Brien PC, Smith GE, Ivnik RJ, et al. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. Neurology 1999;52(7):1397-403.

116.Kabani NJ, Sled JG, Shuper A, Chertkow H. Regional magnetization transfer ratio changes in mild cognitive impairment. Magn Reson Med 2002;47(1):143-8.

117. Arnaiz E, Jelic V, Almkvist O, Wahlund LO, Winblad B, Valind S, et al. Impaired cerebral glucose metabolism and cognitive functioning predict deterioration in mild cognitive impairment. Neuroreport 2001;12(4):851-5.

118.Andreasen N, Minthon L, Vanmechelen E, Vanderstichele H, Davidsson P, Winblad B, et al. Cerebrospinal fluid tau and Abeta42 as predictors of development of Alzheimer's disease in patients with mild cognitive impairment. Neurosci Lett 1999;273(1):5-8.

119.Okamura N, Arai H, Maruyama M, Higuchi M, Matsui T, Tanji H, et al. Combined Analysis of CSF Tau Levels and [(123)I]Iodoamphetamine SPECT in Mild Cognitive Impairment: Implications for a Novel Predictor of Alzheimer's Disease. Am J Psychiatry 2002;159(3):474-6.

120.Milwain E. Mild cognitive impairment: further caution. Lancet 2000;355(9208):1018.

121.Almkvist O, Basun H, Backman L, Herlitz A, Lannfelt L, Small B, et al. Mild cognitive impairment--an early stage of Alzheimer's disease? J Neural Transm Suppl 1998;54:21-9.

122.Fredman L, Magaziner J, Hebel JR, Hawkes W, Zimmerman SI. Depressive symptoms and 6-year mortality among elderly community-dwelling women. Epidemiology 1999;10(1):54-9.

123.Letenneur L, Commenges D, Dartigues JF, Barberger-Gateau P. Incidence of dementia and Alzheimer's disease in elderly community residents of south-western France. Int J Epidemiol 1994;23(6):1256-61.

124.Buhl L, Bojsen-Moller M. Frequency of Alzheimer's disease in a postmortem study of psychiatric patients. Danish Medical Bulletin 1988;35:288-290.

125.Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189-98.

126.Morris JC, Edland S, Clark C, Galasko D, Koss E, Mohs R, et al. The consortium to establish a registry for Alzheimer's disease (CERAD). Part IV. Rates of cognitive change in the longitudinal assessment of probable Alzheimer's disease. Neurology 1993;43(12):2457-65.

127.Geula C, Farlow M., Cummings J., Morris J., Scheltens P., R. A. Alzheimer's disease: Translating neurochemical insights into chemical benefits. Journal of Clinical Psychiatry 2000;61:791-802.

128.Linn RT, Wolf PA, Bachman DL, Knoefel JE, Cobb JL, Belanger AJ, et al. The 'preclinical phase' of probable Alzheimer's disease. A 13-year prospective study of the Framingham cohort. Arch Neurol 1995;52(5):485-90.

129.Small BJ, Viitanen M, Backman L. Mini-Mental State Examination item scores as predictors of Alzheimer's disease: incidence data from the Kungsholmen Project, Stockholm. J Gerontol A Biol Sci Med Sci 1997;52(5):M299-304.

130.Kluger A, Ferris SH, Golomb J, Mittelman MS, Reisberg B. Neuropsychological prediction of decline to dementia in nondemented elderly. J Geriatr Psychiatry Neurol 1999;12(4):168-79.

131.Touchon J, Ritchie K. Prodromal cognitive disorder in Alzheimer's disease. Int J Geriatr Psychiatry 1999;14(7):556-63.

132.Park HL, O'Connell JE, Thomson RG. A systematic review of cognitive decline in the general elderly population. Int J Geriatr Psychiatry 2003;18(12):1121-34.

133.Gurland B, Copeland J, Sharpe L, Kelleher M. The geriatric mental status interview (GMS). Int J Aging Hum Dev 1976;7(4):303-11.

134.Roth M, Tym E, Mountjoy CQ, Huppert FA, Hendrie H, Verma S, et al. CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry 1986;149:698-709.

135.Collie A, Maruff P. An analysis of systems of classifying mild cognitive impairment in older people. Aust N Z J Psychiatry 2002;36(1):133-40.

136.Ganguli M, Seaberg EC, Ratcliff GG, Belle SH, DeKosky ST. Cognitive stability over 2 years in a rural elderly population: the MoVIES project. Neuroepidemiology 1996;15(1):42-50.

137.Kalmijn S, Feskens EJ, Launer LJ, Kromhout D. Polyunsaturated fatty acids, antioxidants, and cognitive function in very old men. Am J Epidemiol 1997;145(1):33-41.

138.Lyketsos CG, Chen LS, Anthony JC. Cognitive decline in adulthood: an 11.5-year follow-up of the Baltimore Epidemiologic Catchment Area study. Am J Psychiatry 1999;156(1):58-65.

139.Dik MG, Deeg DJ, Bouter LM, Corder EH, Kok A, Jonker C. Stroke and apolipoprotein E epsilon4 are independent risk factors for cognitive decline: A population-based study. Stroke 2000;31(10):2431-6.
140.Everson-Rose SA, Mendes de Leon CF, Bienias JL, Wilson RS, Evans DA. Early life conditions and cognitive functioning in later life. Am J Epidemiol 2003;158(11):1083-9.

141.Engelhart MJ, Geerlings MI, Ruitenberg A, Van Swieten JC, Hofman A, Witteman JC, et al. Diet and risk of dementia: Does fat matter?: The Rotterdam Study. Neurology 2002;59(12):1915-21.

142.Johnson-Kozlow M, Kritz-Silverstein D, Barrett-Connor E, Morton D. Coffee consumption and cognitive function among older adults. Am J Epidemiol 2002;156(9):842-50.

143. Teng EL, Chui HC. The Modified Mini-Mental State (3MS) examination. J Clin Psychiatry 1987;48(8):314-8.

144.Yaffe K, Kanaya A, Lindquist K, Simonsick EM, Harris T, Shorr RI, et al. The metabolic syndrome, inflammation, and risk of cognitive decline. Jama 2004;292(18):2237-42.

145.Brandt J, Spencer M, Folstein M. The Telephone Interview for Cognitve Status. Neuropsychiatry Neuropsychology and Behavioral Neurology 1988;1:111-117.

146.Lee S, Kawachi I, Berkman LF, Grodstein F. Education, other socioeconomic indicators, and cognitive function. Am J Epidemiol 2003;157(8):712-20.

147.Wechsler D. A standardized memory scale for clinical use. Journal of Psychology 1945;19:87-95.
148.Wechsler D. WAIS-R manual. New York: Psychological Corporation; 1971.

149.Deeg DJ, Hofman A, van Zonneveld RJ. The association between change in cognitive function and longevity in Dutch elderly. Am J Epidemiol 1990;132(5):973-82.

150.Knopman D, Boland LL, Mosley T, Howard G, Liao D, Szklo M, et al. Cardiovascular risk factors and cognitive decline in middle-aged adults. Neurology 2001;56(1):42-8.

151.Derix MM, Hofstede AB, Teunisse S, Hijdra A, Walstra GJ, Weinstein HC, et al. [CAMDEX-N: the Dutch version of the Cambridge Examination for Mental Disorders of the Elderly with automatic data processing]. Tijdschr Gerontol Geriatr 1991;22(4):143-50.

152. de Koning I, Dippel DW, van Kooten F, Koudstaal PJ. A short screening instrument for poststroke dementia : the R-CAMCOG. Stroke 2000;31(7):1502-8.

153.Pfeiffer E. A short portable mental status questionnaire for the assessment of organic brain deficit in elderly patients. J Am Geriatr Soc 1975;23(10):433-41.

154.Bassuk SS, Berkman LF, Wypij D. Depressive symptomatology and incident cognitive decline in an elderly community sample. Arch Gen Psychiatry 1998;55(12):1073-81.

155.Laursen P. The impact of aging on cognitive functions. An 11 year follow-up study of four age cohorts. Acta Neurol Scand Suppl 1997;172:7-86.

156. Blessed G, Tomlinson BE, Roth M. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. Br J Psychiatry 1968;114(512):797-811.

157.Rey A. L'examen clinique en psychologie. Paris, France: Presse Universitaire de France; 1964.

158.Albert M, Smith LA, Scherr PA, Taylor JO, Evans DA, Funkenstein HH. Use of brief cognitive tests to identify individuals in the community with clinically diagnosed Alzheimer's disease. Int J Neurosci 1991;57(3-4):167-78.

159.Wilson RS, Mendes De Leon CF, Bennett DA, Bienias JL, Evans DA. Depressive symptoms and cognitive decline in a community population of older persons. J Neurol Neurosurg Psychiatry 2004;75(1):126-9.

160.Buschke H, Fuld PA. Evaluating storage, retention, and retrieval in disordered memory and learning. Neurology 1974;24(11):1019-25.

161.Abikoff H, Alvir J, Hong G, Sukoff R, Orazio J, Solomon S, et al. Logical memory subtest of the Wechsler Memory Scale: age and education norms and alternate-form reliability of two scoring systems. J Clin Exp Neuropsychol 1987;9(4):435-48.

162.Muldoon MF, Waldstein SR, Ryan CM, Jennings JR, Polefrone JM, Shapiro AP, et al. Effects of six anti-hypertensive medications on cognitive performance. J Hypertens 2002;20(8):1643-52.

163.Ryan C. Learning and memory deficits in alcoholics. J Stud Alcohol 1980;41(5):437-47.

164.Lezak MD. Neuropsychological assessment, 3rd edition. New York: Oxford University Press; 1995.

165. Russel EW. A multiple scoring method for the assessment of complex memory functions. Journal of Consulting & Clinical Psychology 1975;43:800-809.

166.Richards M, Jarvis MJ, Thompson N, Wadsworth ME. Cigarette smoking and cognitive decline in midlife: evidence from a prospective birth cohort study. Am J Public Health 2003;93(6):994-8.

167.Knopman DS, Ryberg S. A verbal memory test with high predictive accuracy for dementia of the Alzheimer type. Arch Neurol 1989;46(2):141-5.

168.Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 1989;39(9):1159-65.

169. Borkowski JB, Benton AL, Spreen O. Word fluency and brain damage. Neuropsychologia 1967;5:135-140.

170.Lezak MD. Neuropsychological assessment, 2nd edition. New York: Oxford University Press; 1983.

171.Savage RD. Alphabet Coding-Task 15. Perth, Western Australia: Murdoch University; 1984.

172.Smith A. Symbol digit modalities test manual - revised. Los Angeles: Western Psychological Press; 1982.

173.Reitan R. Validity of the Trail-Making Test as an indicator of organic brain disease. Perceptual Motor Skills 1958;8:271-6.

174.Waldstein SR, Giggey PP, Thayer JF, Zonderman AB. Nonlinear relations of blood pressure to cognitive function: the Baltimore Longitudinal Study of Aging. Hypertension 2005;45(3):374-9.

175.Jacobs JW, Bernhard MR, Delgado A, Strain JJ. Screening for organic mental syndromes in the medically ill. Ann Intern Med 1977;86(1):40-6.

176. Salmon DP, Thal LJ, Butters N, Heindel WC. Longitudinal evaluation of dementia of the Alzheimer type: a comparison of 3 standardized mental status examinations. Neurology 1990;40(8):1225-30.

177.Hershey LA, Jaffe DF, Greenough PG, Yang SL. Validation of cognitive and functional assessment instruments in vascular dementia. Int J Psychiatry Med 1987;17(2):183-92.

178.Olsson A, Hoglund K, Sjogren M, Andreasen N, Minthon L, Lannfelt L, et al. Measurement of alpha- and beta-secretase cleaved amyloid precursor protein in cerebrospinal fluid from Alzheimer patients. Exp Neurol 2003;183(1):74-80.

179.Arai H, Nakagawa T, Kosaka Y, Higuchi M, Matsui T, Okamura N, et al. Elevated cerebrospinal fluid tau protein as a predictor of dementia in memory-impaired individuals. Alzheimer Research 1997;3:211-213.

180.Hyman BT, Trojanowski JQ. Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. J Neuropathol Exp Neurol 1997;56(10):1095-7.

181. Selkoe DJ. Physiological production of the beta-amyloid protein and the mechanism of Alzheimer's disease. Trends Neurosci 1993;16(10):403-9.

182.Maruyama M, Arai H, Sugita M, Tanji H, Higuchi M, Okamura N, et al. Cerebrospinal fluid amyloid beta(1-42) levels in the mild cognitive impairment stage of Alzheimer's disease. Exp Neurol 2001;172(2):433-6.

183.Dewey ME, Saz P. Dementia, cognitive impairment and mortality in persons aged 65 and over living in the community: a systematic review of the literature. Int J Geriatr Psychiatry 2001;16(8):751-61.

184.Arve S, Lehtonen A, Tilvis RS. Prognosis of depression with and without dementia in old age. Archives of Gerontology and Geriatrics 1998;27:141-146.

185.Kay DW, Britton PG, Bergmann K, Foster EM. Cognitive function and length of survival in elderly subjects living at home. Aust N Z J Psychiatry 1977;11(2):113-7.

186.Swan GE, Carmelli D, LaRue A. Performance on the digit symbol substitution test and 5-year mortality in the Western Collaborative Group Study. Am J Epidemiol 1995;141(1):32-40.

187.Ostbye T, Hill G, Steenhuis R. Mortality in elderly Canadians with and without dementia: a 5-year follow-up. Neurology 1999;53(3):521-6.

188.Bonaiuto S, Mele M, Galluzzo L, Giannandrea E. Survival and dementia: a 7-year follow-up of an Italian elderly population. Arch Gerontol Geriatr 1995;20(1):105-13.

189. Davidson IA, Dewey ME, Copeland JRM. The relationship between mortality and mental disorder: Evidence from the Liverpool longitudinal study. International Journal of Geriatric Psychiatry 1988;3(2):95-98.

190.Saz P, Launer LJ, JL Diqm, De-La CiqmC, Marcos G, Lobo A. Mortality and mental disorders in a Spanish elderly population. Int J Geriatr Psychiatry 1999;14(12):1031-8.

191.Juva K, Sulkava R, Erkinjunti T, Makela M, Valvanne J, Tilvis R. The prognosis of demented patients: One-year follow-up study of a population sample. International Journal of Geriatric Psychiatry 1994;9:537-541.

192.Meller I, Fichter MM, Schroppel H. Mortality risk in the octo- and nonagenerians: longitudinal results of an epidemiological follow-up community study. Eur Arch Psychiatry Clin Neurosci 1999;249(4):180-9.

193. Evans DA, Smith LA, Scherr PA, Albert MS, Funkenstein HH, Hebert LE. Risk of death from Alzheimer's disease in a community population of older persons. Am J Epidemiol 1991;134(4):403-12.

194.Pavlik VN, de Moraes SA, Szklo M, Knopman DS, Mosley TH, Jr., Hyman DJ. Relation between cognitive function and mortality in middle-aged adults: the atherosclerosis risk in communities study. Am J Epidemiol 2003;157(4):327-34.

195. Farmer ME, Kittner SJ, Rae DS, Bartko JJ, Regier DA. Education and change in cognitive function. The Epidemiologic Catchment Area Study. Ann Epidemiol 1995;5(1):1-7.

196. Elias MF, Elias PK, D'Agostino RB, Silbershatz H, Wolf PA. Role of age, education, and gender on cognitive performance in the Framingham Heart Study: community-based norms. Exp Aging Res 1997;23(3):201-35.

197.Cerhan JR, Folsom AR, Mortimer JA, Shahar E, Knopman DS, McGovern PG, et al. Correlates of cognitive function in middle-aged adults. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Gerontology 1998;44(2):95-105.

198.Evans DA, Hebert LE, Beckett LA, Scherr PA, Albert MS, Chown MJ, et al. Education and other measures of socioeconomic status and risk of incident Alzheimer disease in a defined population of older persons. Arch Neurol 1997;54(11):1399-405.

199. Geerlings MI, Schmand B, Jonker C, Lindeboom J, Bouter LM. Education and incident Alzheimer's disease: a biased association due to selective attrition and use of a two-step diagnostic procedure? Int J Epidemiol 1999;28(3):492-7.

200.Prencipe M, Casini AR, Ferretti C, Lattanzio MT, Fiorelli M, Culasso F. Prevalence of dementia in an elderly rural population: effects of age, sex, and education. J Neurol Neurosurg Psychiatry 1996;60(6):628-33.

201.Adler NE, Boyce WT, Chesney MA, Folkman S, Syme SL. Socioeconomic inequalities in health. No easy solution. Jama 1993;269(24):3140-5.

202.Staff RT, Murray AD, Deary IJ, Whalley LJ. What provides cerebral reserve? Brain 2004;127(Pt 5):1191-9.

203.Kaplan GA, Turrell G, Lynch JW, Everson SA, Helkala EL, Salonen JT. Childhood socioeconomic position and cognitive function in adulthood. Int J Epidemiol 2001;30(2):256-63.

204. Albert MS. How does education affect cognitive function? Ann Epidemiol 1995;5(1):76-8.

205.Wilson RS, Bennett DA, Beckett LA, Morris MC, Gilley DW, Bienias JL, et al. Cognitive activity in older persons from a geographically defined population. J Gerontol B Psychol Sci Soc Sci 1999;54(3):P155-60.

206.Cobb JL, Wolf PA, Au R, White R, D'Agostino RB. The effect of education on the incidence of dementia and Alzheimer's disease in the Framingham Study. Neurology 1995;45(9):1707-12.

207.Helmer C, Letenneur L, Rouch I, Richard-Harston S, Barberger-Gateau P, Fabrigoule C, et al. Occupation during life and risk of dementia in French elderly community residents. J Neurol Neurosurg Psychiatry 2001;71(3):303-9.

208. Bassuk SS, Glass TA, Berkman LF. Social disengagement and incident cognitive decline in community-dwelling elderly persons. Ann Intern Med 1999;131(3):165-73.

209. Wilson RS, Schneider JA, Barnes LL, Beckett LA, Aggarwal NT, Cochran EJ, et al. The apolipoprotein E epsilon 4 allele and decline in different cognitive systems during a 6-year period. Arch Neurol 2002;59(7):1154-60.

210.Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261(5123):921-3.

211. Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43(8):1467-72.

212.Hsiung GY, Sadovnick AD, Feldman H. Apolipoprotein E epsilon4 genotype as a risk factor for cognitive decline and dementia: data from the Canadian Study of Health and Aging. Cmaj 2004;171(8):863-7.

213.Kalmijn S, Feskens EJ, Launer LJ, Kromhout D. Cerebrovascular disease, the apolipoprotein e4 allele, and cognitive decline in a community-based study of elderly men. Stroke 1996;27(12):2230-5.

214. Kim KW, Youn JC, Jhoo JH, Lee DY, Lee KU, Lee JH, et al. Apolipoprotein E epsilon 4 allele is not associated with the cognitive impairment in community-dwelling normal elderly individuals. Int J Geriatr Psychiatry 2002;17(7):635-40.

215.Yip AG, Brayne C, Easton D, Rubinsztein DC. Apolipoprotein E4 is only a weak predictor of dementia and cognitive decline in the general population. J Med Genet 2002;39(9):639-43.

216.Blair CK, Folsom AR, Knopman DS, Bray MS, Mosley TH, Boerwinkle E. APOE genotype and cognitive decline in a middle-aged cohort. Neurology 2005;64(2):268-76.

217. Gibbs RB, Aggarwal P. Estrogen and basal forebrain cholinergic neurons: implications for brain aging and Alzheimer's disease-related cognitive decline. Horm Behav 1998;34(2):98-111.

218.McEwen BS, Alves SE. Estrogen actions in the central nervous system. Endocr Rev 1999;20(3):279-307.

219.Solerte SB, Fioravanti M, Racchi M, Trabucchi M, Zanetti O, Govoni S. Menopause and estrogen deficiency as a risk factor in dementing illness: hypothesis on the biological basis. Maturitas 1999;31(2):95-101.

220.Monk D, Brodaty H. Use of estrogens for the prevention and treatment of Alzheimer's disease. Dement Geriatr Cogn Disord 2000;11(1):1-10.

221.Geerlings MI, Ruitenberg A, Witteman JC, van Swieten JC, Hofman A, van Duijn CM, et al. Reproductive period and risk of dementia in postmenopausal women. Jama 2001;285(11):1475-81.

222.Paganini-Hill A, Henderson VW. Estrogen deficiency and risk of Alzheimer's disease in women. Am J Epidemiol 1994;140(3):256-61.

223.Kawas C, Resnick S, Morrison A, Brookmeyer R, Corrada M, Zonderman A, et al. A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging. Neurology 1997;48(6):1517-21.

224.Tang MX, Jacobs D, Stern Y, Marder K, Schofield P, Gurland B, et al. Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 1996;348(9025):429-32.

225.LeBlanc ES, Janowsky J, Chan BK, Nelson HD. Hormone replacement therapy and cognition: systematic review and meta-analysis. Jama 2001;285(11):1489-99.

226.Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, et al. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. Jama 2004;291(24):2947-58.

227.Herbert LE, Scherr PA, Beckett LA, Albert MS, Rosner B, Taylor JO, et al. Relation of smoking and low-to-moderate alcohol consumption to change in cognitive function: a longitudinal study in a defined community of older persons. Am J Epidemiol 1993;137(8):881-91.

228.Ford AB, Mefrouche Z, Friedland RP, Debanne SM. Smoking and cognitive impairment: a population-based study. J Am Geriatr Soc 1996;44(8):905-9.

229.Edelstein SL, Kritz-Silverstein D, Barrett-Connor E. Prospective association of smoking and alcohol use with cognitive function in an elderly cohort. J Womens Health 1998;7(10):1271-81.

230.Wang HX, Fratiglioni L, Frisoni GB, Viitanen M, Winblad B. Smoking and the occurrence of Alzheimer's disease: cross-sectional and longitudinal data in a population-based study. Am J Epidemiol 1999;149(7):640-4.

231.Doll R, Peto R, Boreham J, Sutherland I. Smoking and dementia in male British doctors: prospective study. Bmj 2000;320(7242):1097-102.

232. Ott A, Slooter AJ, Hofman A, van Harskamp F, Witteman JC, Van Broeckhoven C, et al. Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study. Lancet 1998;351(9119):1840-3.

233.Launer LJ, Andersen K, Dewey ME, Letenneur L, Ott A, Amaducci LA, et al. Rates and risk factors for dementia and Alzheimer's disease: results from EURODEM pooled analyses. EURODEM Incidence Research Group and Work Groups. European Studies of Dementia. Neurology 1999;52(1):78-84.

234.Cervilla JA, Prince M, Mann A. Smoking, drinking, and incident cognitive impairment: a cohort community based study included in the Gospel Oak project. J Neurol Neurosurg Psychiatry 2000;68(5):622-6.

235.Kalmijn S, van Boxtel MP, Verschuren MW, Jolles J, Launer LJ. Cigarette smoking and alcohol consumption in relation to cognitive performance in middle age. Am J Epidemiol 2002;156(10):936-44.

236.Hendrie HC, Gao S, Hall KS, Hui SL, Unverzagt FW. The relationship between alcohol consumption, cognitive performance, and daily functioning in an urban sample of older black Americans. J Am Geriatr Soc 1996;44(10):1158-65.

237.Kilander L, Nyman H, Boberg M, Lithell H. Cognitive function, vascular risk factors and education. A cross-sectional study based on a cohort of 70-year-old men. J Intern Med 1997;242(4):313-21.

238.Dufouil C, Ducimetiere P, Alperovitch A. Sex differences in the association between alcohol consumption and cognitive performance. EVA Study Group. Epidemiology of Vascular Aging. Am J Epidemiol 1997;146(5):405-12.

239.Christian JC, Reed T, Carmelli D, Page WF, Norton JA, Jr., Breitner JC. Self-reported alcohol intake and cognition in aging twins. J Stud Alcohol 1995;56(4):414-6.

240.Elwood PC, Gallacher JE, Hopkinson CA, Pickering J, Rabbitt P, Stollery B, et al. Smoking, drinking, and other life style factors and cognitive function in men in the Caerphilly cohort. J Epidemiol Community Health 1999;53(1):9-14.

241.James JE. Caffeine & health. London, England: Academic Press; 1991.

242.Barrone JJ, Roberts HJ, editors. Human consumption of caffeine. Berlin, Germany: Springer-Verlag; 1984.

243.Lienert GA, Huber HP. Differential effects of coffee on speed and power tests. J Psychol 1966;63(2):269-74.

244.Battig K, Buzzi R. Effect of coffee on the speed of subject-paced information processing. Neuropsychobiology 1986;16(2-3):126-30.

245.Smith AP, Brockman P, Flynn R, Maben A, Thomas M. Investigation of the effects of coffee on alertness and performance during the day and night. Neuropsychobiology 1993;27(4):217-23.

246.Riedel W, Hogervorst E, Leboux R, Verhey F, van Praag H, Jolles J. Caffeine attenuates scopolamine-induced memory impairment in humans. Psychopharmacology (Berl) 1995;122(2):158-68.

247.Broe GA, Henderson AS, Creasey H, McCusker E, Korten AE, Jorm AF, et al. A case-control study of Alzheimer's disease in Australia. Neurology 1990;40(11):1698-707.

248.Li G, Shen YC, Chen CH, Zhau YW, Li SR, Lu M. A three-year follow-up study of age-related dementia in an urban area of Beijing. Acta Psychiatr Scand 1991;83(2):99-104.

249.Yoshitake T, Kiyohara Y, Kato I, Ohmura T, Iwamoto H, Nakayama K, et al. Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly Japanese population: the Hisayama Study. Neurology 1995;45(6):1161-8.

250.Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K. Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 2001;58(3):498-504.

251.Yaffe K, Barnes D, Nevitt M, Lui LY, Covinsky K. A prospective study of physical activity and cognitive decline in elderly women: women who walk. Arch Intern Med 2001;161(14):1703-8.

252. van Gelder BM, Tijhuis MA, Kalmijn S, Giampaoli S, Nissinen A, Kromhout D. Physical activity in relation to cognitive decline in elderly men: the FINE Study. Neurology 2004;63(12):2316-21.

253.Weuve J, Kang JH, Manson JE, Breteler MM, Ware JH, Grodstein F. Physical activity, including walking, and cognitive function in older women. Jama 2004;292(12):1454-61.

254.Rogers RL, Meyer JS, Mortel KF. After reaching retirement age physical activity sustains cerebral perfusion and cognition. J Am Geriatr Soc 1990;38(2):123-8.

255.Dustman RE, Ruhling RO, Russell EM, Shearer DE, Bonekat HW, Shigeoka JW, et al. Aerobic exercise training and improved neuropsychological function of older individuals. Neurobiol Aging 1984;5(1):35-42.

256.Spirduso WW. Physical fitness, aging, and psychomotor speed: a review. J Gerontol 1980;35(6):850-65.

257.Gomez-Pinilla F, Dao L, So V. Physical exercise induces FGF-2 and its mRNA in the hippocampus. Brain Res 1997;764(1-2):1-8.

258.Cotman CW, Engesser-Cesar C. Exercise enhances and protects brain function. Exerc Sport Sci Rev 2002;30(2):75-9.

259.Kretsch MJ, Green MW, Fong AK, Elliman NA, Johnson HL. Cognitive effects of a long-term weight reducing diet. Int J Obes Relat Metab Disord 1997;21(1):14-21.

260.Green MW, Rogers PJ. Impaired cognitive functioning during spontaneous dieting. Psychol Med 1995;25(5):1003-10.

261.Barrett-Connor E, Edelstein SL, Corey-Bloom J, Wiederholt WC. Weight loss precedes dementia in community-dwelling older adults. J Am Geriatr Soc 1996;44(10):1147-52.

262.Bryan J, Tiggemann M. The effect of weight-loss dieting on cognitive performance and psychological well-being in overweight women. Appetite 2001;36(2):147-56.

263.Wolf-Klein GP, Silverstone FA, Levy AP. Nutritional patterns and weight change in Alzheimer patients. Int Psychogeriatr 1992;4(1):103-18.

264. Brubacher D, Monsch AU, Stahelin HB. Weight change and cognitive performance. Int J Obes Relat Metab Disord 2004;28(9):1163-7.

265.Behl C. Amyloid beta-protein toxicity and oxidative stress in Alzheimer's disease. Cell Tissue Res 1997;290(3):471-80.

266. Christen Y. Oxidative stress and Alzheimer disease. Am J Clin Nutr 2000;71(2):621S-629S.
267.Grundman M. Vitamin E and Alzheimer disease: the basis for additional clinical trials. Am J Clin Nutr 2000;71(2):630S-636S.

268.Morris MC, Beckett, L. A., Scherr, P. A., et al. Vitamin E and C supplement use and risk of incident Alzheimer disease. Alzheimer Disease Association Disorders 1998;12:121-126.

269.Zandi PP, Anthony JC, Khachaturian AS, Stone SV, Gustafson D, Tschanz JT, et al. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. Arch Neurol 2004;61(1):82-8.

270.Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, et al. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. Neurology 2000;54(6):1265-72.

271.Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues JF. Intake of flavonoids and risk of dementia. Eur J Epidemiol 2000;16(4):357-63.

272.Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, et al. Dietary intake of antioxidants and risk of Alzheimer disease. Jama 2002;287(24):3223-9.

273.Morris MC. Diet and Alzheimer's disease: what the evidence shows. MedGenMed 2004;6(1):48.

274.Luchsinger JA, Tang MX, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. Arch Neurol 2003;60(2):203-8.

275.Laurin D, Masaki KH, Foley DJ, White LR, Launer LJ. Midlife dietary intake of antioxidants and risk of late-life incident dementia: the Honolulu-Asia Aging Study. Am J Epidemiol 2004;159(10):959-67.

276. Helmer C, Peuchant E, Letenneur L, Bourdel-Marchasson I, Larrieu S, Dartigues JF, et al. Association between antioxidant nutritional indicators and the incidence of dementia: results from the PAQUID prospective cohort study. Eur J Clin Nutr 2003;57(12):1555-61.

277. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Med 1998;49:31-62.

278.Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55(11):1449-55.

279.Nilsson K, Gustafson L, Faldt R, Andersson A, Brattstrom L, Lindgren A, et al. Hyperhomocysteinaemia--a common finding in a psychogeriatric population. Eur J Clin Invest 1996;26(10):853-9.

280.Joosten E, Lesaffre E, Riezler R, Ghekiere V, Dereymaeker L, Pelemans W, et al. Is metabolic evidence for vitamin B-12 and folate deficiency more frequent in elderly patients with Alzheimer's disease? J Gerontol A Biol Sci Med Sci 1997;52(2):M76-9.

281.Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346(7):476-83.

282.Miller JW, Green R, Ramos MI, Allen LH, Mungas DM, Jagust WJ, et al. Homocysteine and cognitive function in the Sacramento Area Latino Study on Aging. Am J Clin Nutr 2003;78(3):441-7.

283.Kalmijn S, Launer LJ, Lindemans J, Bots ML, Hofman A, Breteler MM. Total homocysteine and cognitive decline in a community-based sample of elderly subjects: the Rotterdam Study. Am J Epidemiol 1999;150(3):283-9.

284.Breteler MM. Vascular involvement in cognitive decline and dementia. Epidemiologic evidence from the Rotterdam Study and the Rotterdam Scan Study. Ann N Y Acad Sci 2000;903:457-65.

285.Rao R. Cerebrovascular disease and late life depression: an age old association revisited. Int J Geriatr Psychiatry 2000;15(5):419-33.

286.Riggs KM, Spiro A, 3rd, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. Am J Clin Nutr 1996;63(3):306-14.

287. Launer LJ, Masaki K, Petrovitch H, Foley D, Havlik RJ. The association between midlife blood pressure levels and late-life cognitive function. The Honolulu-Asia Aging Study. Jama 1995;274(23):1846-51.

288.Breteler MM, Claus JJ, Grobbee DE, Hofman A. Cardiovascular disease and distribution of cognitive function in elderly people: the Rotterdam Study. Bmj 1994;308(6944):1604-8.

289.Phillips NA, Mate-Kole CC. Cognitive deficits in peripheral vascular disease. A comparison of mild stroke patients and normal control subjects. Stroke 1997;28(4):777-84.

290. Ott A, Stolk RP, Hofman A, van Harskamp F, Grobbee DE, Breteler MM. Association of diabetes mellitus and dementia: the Rotterdam Study. Diabetologia 1996;39(11):1392-7.

291.Slooter AJ, Tang MX, van Duijn CM, Stern Y, Ott A, Bell K, et al. Apolipoprotein E epsilon4 and the risk of dementia with stroke. A population-based investigation. Jama 1997;277(10):818-21.

292.Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Arch Neurol 2004;61(5):661-6.

293.Yaffe K, Blackwell T, Kanaya AM, Davidowitz N, Barrett-Connor E, Krueger K. Diabetes, impaired fasting glucose, and development of cognitive impairment in older women. Neurology 2004;63(4):658-63.

294.Fontbonne A, Berr C, Ducimetiere P, Alperovitch A. Changes in cognitive abilities over a 4-year period are unfavorably affected in elderly diabetic subjects: results of the Epidemiology of Vascular Aging Study. Diabetes Care 2001;24(2):366-70.

295.Hassing LB, Hofer SM, Nilsson SE, Berg S, Pedersen NL, McClearn G, et al. Comorbid type 2 diabetes mellitus and hypertension exacerbates cognitive decline: evidence from a longitudinal study. Age Ageing 2004;33(4):355-61.

296.Burns M, Duff K. Cholesterol in Alzheimer's disease and tauopathy. Ann N Y Acad Sci 2002;977:367-75.

297.Scheltens P, Kittner B. Preliminary results from an MRI/CT-based database for vascular dementia and Alzheimer's disease. Ann N Y Acad Sci 2000;903:542-6.

298. Kuriyama M, Takahashi K, Yamano T, Hokezu Y, Togo S, Osame M, et al. Low levels of serum apolipoprotein A I and A II in senile dementia. Jpn J Psychiatry Neurol 1994;48(3):589-93.

299.Muckle TJ, Roy JR. High-density lipoprotein cholesterol in differential diagnosis of senile dementia. Lancet 1985;1(8439):1191-3.

300.Kuriyama M, Hokezu Y, Togo S, Nagata K, Takahashi K, Igakura T, et al. [Serum lipids, lipoproteins and apolipoproteins in patients with senile dementia]. Nippon Ronen Igakkai Zasshi 1992;29(7-8):559-64.

301.Klich-Raczka A, Necki M, Wizner B, Baron T, Adamkiewicz-Piejko A, Gryglewska B, et al. [Vascular dementia and systemic changes]. Przegl Lek 2002;59(4-5):269-71.

302.Wieringa GE, Burlinson S, Rafferty JA, Gowland E, Burns A. Apolipoprotein E genotypes and serum lipid levels in Alzheimer's disease and multi-infarct dementia. Int J Geriatr Psychiatry 1997;12(3):359-62.

303. Scacchi R, De Bernardini L, Mantuano E, Vilardo T, Donini LM, Ruggeri M, et al. DNA polymorphisms of apolipoprotein B and angiotensin I-converting enzyme genes and relationships with lipid levels in Italian patients with vascular dementia or Alzheimer's disease. Dement Geriatr Cogn Disord 1998;9(4):186-90.

304.Lesser G, Kandiah K, Libow LS, Likourezos A, Breuer B, Marin D, et al. Elevated serum total and LDL cholesterol in very old patients with Alzheimer's disease. Dement Geriatr Cogn Disord 2001;12(2):138-45.

305.Kuo YM, Emmerling MR, Bisgaier CL, Essenburg AD, Lampert HC, Drumm D, et al. Elevated low-density lipoprotein in Alzheimer's disease correlates with brain abeta 1-42 levels. Biochem Biophys Res Commun 1998;252(3):711-5.

306.Michikawa M. Cholesterol paradox: is high total or low HDL cholesterol level a risk for Alzheimer's disease? J Neurosci Res 2003;72(2):141-6.

307.Reitz C, Tang MX, Luchsinger J, Mayeux R. Relation of plasma lipids to Alzheimer disease and vascular dementia. Arch Neurol 2004;61(5):705-14.

308. Launer LJ. Demonstrating the case that AD is a vascular disease: epidemiologic evidence. Ageing Res Rev 2002;1(1):61-77.

309.Kalmijn S, Feskens EJ, Launer LJ, Stijnen T, Kromhout D. Glucose intolerance, hyperinsulinaemia and cognitive function in a general population of elderly men. Diabetologia 1995;38(9):1096-102.

310.Gregg EW, Yaffe K, Cauley JA, Rolka DB, Blackwell TL, Narayan KM, et al. Is diabetes associated with cognitive impairment and cognitive decline among older women? Study of Osteoporotic Fractures Research Group. Arch Intern Med 2000;160(2):174-80.

311.Wilson RS, Barnes LL, Mendes de Leon CF, Aggarwal NT, Schneider JS, Bach J, et al. Depressive symptoms, cognitive decline, and risk of AD in older persons. Neurology 2002;59(3):364-70.

312. Prince M, Lewis G, Bird A, Blizard R, Mann A. A longitudinal study of factors predicting change in cognitive test scores over time, in an older hypertensive population. Psychol Med 1996;26(3):555-68.

313. Yaffe K, Blackwell T, Gore R, Sands L, Reus V, Browner WS. Depressive symptoms and cognitive decline in nondemented elderly women: a prospective study. Arch Gen Psychiatry 1999;56(5):425-30.

314. Dufouil C, Fuhrer R, Dartigues JF, Alperovitch A. Longitudinal analysis of the association between depressive symptomatology and cognitive deterioration. Am J Epidemiol 1996;144(7):634-41.

315.Henderson AS, Korten AE, Jacomb PA, Mackinnon AJ, Jorm AF, Christensen H, et al. The course of depression in the elderly: a longitudinal community-based study in Australia. Psychol Med 1997;27(1):119-29.

316.Geerlings MI, Schoevers RA, Beekman AT, Jonker C, Deeg DJ, Schmand B, et al. Depression and risk of cognitive decline and Alzheimer's disease. Results of two prospective community-based studies in The Netherlands. Br J Psychiatry 2000;176:568-75.

317.McShane R, Keene J, Gedling K, Fairburn C, Jacoby R, Hope T. Do neuroleptic drugs hasten cognitive decline in dementia? Prospective study with necropsy follow up. Bmj 1997;314(7076):266-70.

318.Podewils LJ, Lyketsos CG. Tricyclic antidepressants and cognitive decline. Psychosomatics 2002;43(1):31-5.

319.de Craen AJ, Gussekloo J, Vrijsen B, Westendorp RG. Meta-analysis of nonsteroidal antiinflammatory drug use and risk of dementia. Am J Epidemiol 2005;161(2):114-20.

320.Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. Arch Neurol 2000;57(10):1439-43.

321.Hajjar I, Schumpert J, Hirth V, Wieland D, Eleazer GP. The impact of the use of statins on the prevalence of dementia and the progression of cognitive impairment. J Gerontol A Biol Sci Med Sci 2002;57(7):M414-8.

322.Amenta F, Mignini F, Rabbia F, Tomassoni D, Veglio F. Protective effect of anti-hypertensive treatment on cognitive function in essential hypertension: analysis of published clinical data. J Neurol Sci 2002;203-204:147-51.

323.Brady LM, Williams CM, Lovegrove JA. Dietary PUFA and the metabolic syndrome in Indian Asians living in the UK. Proc Nutr Soc 2004;63(1):115-25.

324. Whelton SP, He J, Whelton PK, Muntner P. Meta-analysis of observational studies on fish intake and coronary heart disease. Am J Cardiol 2004;93(9):1119-23.

325.He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation 2004;109(22):2705-11.

326.Wang L, Folsom AR, Eckfeldt JH. Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Nutr Metab Cardiovasc Dis 2003;13(5):256-66.

327. He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, et al. Fish consumption and incidence of stroke: a meta-analysis of cohort studies. Stroke 2004;35(7):1538-42.

328.Mozaffarian D, Longstreth WT, Jr., Lemaitre RN, Manolio TA, Kuller LH, Burke GL, et al. Fish consumption and stroke risk in elderly individuals: the cardiovascular health study. Arch Intern Med 2005;165(2):200-6.

329.Iso H, Sato S, Umemura U, Kudo M, Koike K, Kitamura A, et al. Linoleic acid, other fatty acids, and the risk of stroke. Stroke 2002;33(8):2086-93.

330. Zheng ZJ, Folsom AR, Ma J, Arnett DK, McGovern PG, Eckfeldt JH. Plasma fatty acid composition and 6-year incidence of hypertension in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 1999;150(5):492-500.

331.Grimsgaard S, Bonaa KH, Jacobsen BK, Bjerve KS. Plasma saturated and linoleic fatty acids are independently associated with blood pressure. Hypertension 1999;34(3):478-83.

332.Shahar E, Folsom AR, Wu KK, Dennis BH, Shimakawa T, Conlan MG, et al. Associations of fish intake and dietary n-3 polyunsaturated fatty acids with a hypocoagulable profile. The Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler Thromb 1993;13(8):1205-12.

333.Hostmark AT, Bjerkedal T, Kierulf P, Flaten H, Ulshagen K. Fish oil and plasma fibrinogen. Bmj 1988;297(6642):180-1.

334.Radack K, Deck C, Huster G. The comparative effects of n-3 and n-6 polyunsaturated fatty acids on plasma fibrinogen levels: a controlled clinical trial in hypertriglyceridemic subjects. J Am Coll Nutr 1990;9(4):352-7.

335.Haglund O, Wallin R, Luostarinen R, Saldeen T. Effects of a new fluid fish oil concentrate, ESKIMO-3, on triglycerides, cholesterol, fibrinogen and blood pressure. J Intern Med 1990;227(5):347-53.

336.Conquer JA, Cheryk LA, Chan E, Gentry PA, Holub BJ. Effect of supplementation with dietary seal oil on selected cardiovascular risk factors and hemostatic variables in healthy male subjects. Thromb Res 1999;96(3):239-50.

337.Mortensen JZ, Schmidt EB, Nielsen AH, Dyerberg J. The effect of N-6 and N-3 polyunsaturated fatty acids on hemostasis, blood lipids and blood pressure. Thromb Haemost 1983;50(2):543-6.

338.Marckmann P, Jespersen J, Leth T, Sandstrom B. Effect of fish diet versus meat diet on blood lipids, coagulation and fibrinolysis in healthy young men. J Intern Med 1991;229(4):317-23.

339. Archer SL, Green D, Chamberlain M, Dyer AR, Liu K. Association of dietary fish and n-3 fatty acid intake with hemostatic factors in the coronary artery risk development in young adults (CARDIA) study. Arterioscler Thromb Vasc Biol 1998;18(7):1119-23.

340. Feskens EJ, Bowles CH, Kromhout D. Inverse association between fish intake and risk of glucose intolerance in normoglycemic elderly men and women. Diabetes Care 1991;14(11):935-41.

341. Feskens EJ, Virtanen SM, Rasanen L, Tuomilehto J, Stengard J, Pekkanen J, et al. Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. Diabetes Care 1995;18(8):1104-12.

342.Fasching P, Ratheiser K, Waldhausl W, Rohac M, Osterrode W, Nowotny P, et al. Metabolic effects of fish-oil supplementation in patients with impaired glucose tolerance. Diabetes 1991;40(5):583-9.

343.Popp-Snijders C, Schouten JA, Heine RJ, van der Meer J, van der Veen EA. Dietary supplementation of omega-3 polyunsaturated fatty acids improves insulin sensitivity in non-insulin-dependent diabetes. Diabetes Res 1987;4(3):141-7.

344. Storlien LH, Pan DA, Kriketos AD, O'Connor J, Caterson ID, Cooney GJ, et al. Skeletal muscle membrane lipids and insulin resistance. Lipids 1996;31 Suppl:S261-5.

345.Schmidt EB, Varming K, Ernst E, Madsen P, Dyerberg J. Dose-response studies on the effect of n-3 polyunsaturated fatty acids on lipids and haemostasis. Thromb Haemost 1990;63(1):1-5.

346.Harris WS, Windsor SL, Dujovne CA. Effects of four doses of n-3 fatty acids given to hyperlipidemic patients for six months. J Am Coll Nutr 1991;10(3):220-7.

347.Brown AJ, Roberts DC. Moderate fish oil intake improves lipemic response to a standard fat meal. A study in 25 healthy men. Arterioscler Thromb 1991;11:457-466.

348.Zampelas A, Williams CM, Morgan LM, Wright J, Quinlan PT. The effect of triacylglycerol fatty acid positional distribution on postprandial plasma metabolite and hormone responses in normal adult men. Br J Nutr 1994;71(3):401-10.

349. Nestel PJ. Fish oil and cardiovascular disease: lipids and arterial function. Am J Clin Nutr 2000;71(1 Suppl):228S-31S.

350.Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 2004;79(6):935-45.
351.Hibbeln JR, Salem N, Jr. Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. Am J Clin Nutr 1995;62(1):1-9.

352.Hibbeln JR, Umhau JC, George DT, Salem N, Jr. Do plasma polyunsaturates predict hostility and depression? World Rev Nutr Diet 1997;82:175-86.

353. Adams PB, Lawson S, Sanigorski A, Sinclair AJ. Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. Lipids 1996;31 Suppl:S157-61.

354.Maes M, Smith R, Christophe A, Cosyns P, Desnyder R, Meltzer H. Fatty acid composition in major depression: decreased omega 3 fractions in cholesteryl esters and increased C20: 4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids. J Affect Disord 1996;38(1):35-46.

355.Edwards R, Peet M, Shay J, Horrobin D. Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. J Affect Disord 1998;48(2-3):149-55.

356.Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. Biol Psychiatry 1998;43(5):315-9.

357.Shahar E, Folsom AR, Melnick SL, Tockman MS, Comstock GW, Gennaro V, et al. Dietary n-3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. Atherosclerosis Risk in Communities Study Investigators. N Engl J Med 1994;331(4):228-33.

358.Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 1965;17(5):281-95.

359.Keys A. Serum cholesterol response to dietary cholesterol. Am J Clin Nutr 1984;40(2):351-9.

360.Arntzenius AC, Kromhout D, Barth JD, Reiber JH, Bruschke AV, Buis B, et al. Diet, lipoproteins, and the progression of coronary atherosclerosis. The Leiden Intervention Trial. N Engl J Med 1985;312(13):805-11.

361.Katan MB, Beynen AC, de Vries JH, Nobels A. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. Am J Epidemiol 1986;123(2):221-34.

362. Kushi LH, Samonds KW, Lacey JM, Brown PT, Bergan JG, Sacks FM. The association of dietary fat with serum cholesterol in vegetarians: the effect of dietary assessment on the correlation coefficient. Am J Epidemiol 1988;128(5):1054-64.

363.Willet WC. Nutritional epidemiology 2nd ed. New York: Oxford University Press; 1998.
364.Holman RT. Control of polyunsaturated acids in tissue lipids. J Am Coll Nutr 1986;5(2):183-211.

365.Silverman DI, Reis GJ, Sacks FM, Boucher TM, Pasternak RC. Usefulness of plasma phospholipid N-3 fatty acid levels in predicting dietary fish intake in patients with coronary artery disease. Am J Cardiol 1990;66(10):860-2.

366.London SJ, Sacks FM, Caesar J, Stampfer MJ, Siguel E, Willett WC. Fatty acid composition of subcutaneous adipose tissue and diet in postmenopausal US women. Am J Clin Nutr 1991;54(2):340-5.

367.Hunter DJ, Rimm EB, Sacks FM, Stampfer MJ, Colditz GA, Litin LB, et al. Comparison of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. Am J Epidemiol 1992;135(4):418-27.

368.Tjonneland A, Overvad K, Thorling E, Ewertz M. Adipose tissue fatty acids as biomarkers of dietary exposure in Danish men and women. Am J Clin Nutr 1993;57(5):629-33.

369.Feunekes GI, Van Staveren WA, De Vries JH, Burema J, Hautvast JG. Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. Am J Clin Nutr 1993;58(4):489-96.

370.Kardinaal AF, van 't Veer P, Brants HA, van den Berg H, van Schoonhoven J, Hermus RJ. Relations between antioxidant vitamins in adipose tissue, plasma, and diet. Am J Epidemiol 1995;141(5):440-50.

371. Ma J, Folsom AR, Shahar E, Eckfeldt JH. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Am J Clin Nutr 1995;62(3):564-71.

372.Andersen LF, Solvoll K, Drevon CA. Very-long-chain n-3 fatty acids as biomarkers for intake of fish and n-3 fatty acid concentrates. Am J Clin Nutr 1996;64(3):305-11.

373. Hjartaker A, Lund E, Bjerve KS. Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire. Eur J Clin Nutr 1997;51(11):736-42.

374.Kobayashi M, Sasaki S, Kawabata T, Hasegawa K, Akabane M, Tsugane S. Single measurement of serum phospholipid fatty acid as a biomarker of specific fatty acid intake in middle-aged Japanese men. Eur J Clin Nutr 2001;55(8):643-50.

375.Brunner E, Stallone D, Juneja M, Bingham S, Marmot M. Dietary assessment in Whitehall II: comparison of 7 d diet diary and food-frequency questionnaire and validity against biomarkers. Br J Nutr 2001;86(3):405-14.

376.Baylin A, Kabagambe EK, Siles X, Campos H. Adipose tissue biomarkers of fatty acid intake. Am J Clin Nutr 2002;76(4):750-7.

377.Knutsen SF, Fraser GE, Beeson WL, Lindsted KD, Shavlik DJ. Comparison of adipose tissue fatty acids with dietary fatty acids as measured by 24-hour recall and food frequency questionnaire in Black and White Adventists: the Adventist Health Study. Ann Epidemiol 2003;13(2):119-27.

378. Patch C, Murphy K, Mansour J, Tapsel L, Meyer B, Mori T, et al. Erythrocyte biomarker-based validation of a diet history method used in a dietary intervention trial. Asia Pac J Clin Nutr 2004;13(Suppl):S60.

379.Cantwell MM, Gibney MJ, Cronin D, Younger KM, O'Neill JP, Hogan L, et al. Development and validation of a food-frequency questionnaire for the determination of detailed fatty acid intakes. Public Health Nutr 2005;8(1):97-107.

380.Marckmann P, Lassen A, Haraldsdottir J, Sandstrom B. Biomarkers of habitual fish intake in adipose tissue. Am J Clin Nutr 1995;62(5):956-9.

381. Lands WE. Long-term fat intake and biomarkers. Am J Clin Nutr 1995;61(3 Suppl):721S-725S.

382.Lands WE, Libelt B, Morris A, Kramer NC, Prewitt TE, Bowen P, et al. Maintenance of lower proportions of (n - 6) eicosanoid precursors in phospholipids of human plasma in response to added dietary (n - 3) fatty acids. Biochim Biophys Acta 1992;1180(2):147-62.

383. Lands WE, Morris A, Libelt B. Quantitative effects of dietary polyunsaturated fats on the composition of fatty acids in rat tissues. Lipids 1990;25(9):505-16.

384.Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids 2000;35(12):1305-12.

385.Tully AM, Roche HM, Doyle R, Fallon C, Bruce I, Lawlor B, et al. Low serum cholesteryl ester-docosahexaenoic acid levels in Alzheimer's disease: a case-control study. Br J Nutr 2003;89(4):483-9.

386.Heude B, Ducimetiere P, Berr C. Cognitive decline and fatty acid composition of erythrocyte membranes--The EVA Study. Am J Clin Nutr 2003;77(4):803-8.

387. Laurin D, Verreault, R, Lindsay, J, Dewailly, E, Holub, BJ. Omega-3 fatty acids and risk of cognitive impairment and dementia. J Alzheimers Dis 2003;5(4):315-22.

388.Kalmijn S, van Boxtel MP, Ocke M, Verschuren WM, Kromhout D, Launer LJ. Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. Neurology 2004;62(2):275-80.

389.Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60(7):940-6.

390.Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42(5):776-82.

391.Kilander L, Nyman H, Boberg M, Hansson L, Lithell H. Hypertension is related to cognitive impairment: a 20-year follow-up of 999 men. Hypertension 1998;31(3):780-6.

392. Swan GE, Carmelli D, Larue A. Systolic blood pressure tracking over 25 to 30 years and cognitive performance in older adults. Stroke 1998;29(11):2334-40.

393.Glynn RJ, Beckett LA, Hebert LE, Morris MC, Scherr PA, Evans DA. Current and remote blood pressure and cognitive decline. Jama 1999;281(5):438-45.

394.Harrington F, Saxby BK, McKeith IG, Wesnes K, Ford GA. Cognitive performance in hypertensive and normotensive older subjects. Hypertension 2000;36(6):1079-82.

395. Stewart R, Prince M, Mann A. Age, vascular risk, and cognitive decline in an older, British, African-Caribbean population. J Am Geriatr Soc 2003;51(11):1547-53.

396.Maritim AC, Sanders RA, Watkins JB, 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003;17(1):24-38.

397.Wada H, Hagiwara SI, Saitoh E, Ieki R, Okamura T, Ota T, et al. Increased oxidative stress in patients with chronic obstructive pulmonary disease (COPD) as measured by redox status of plasma coenzyme Q(10). Pathophysiology 2005.

398. Shea TB, Rogers E, Ashline D, Ortiz D, Sheu MS. Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue. Free Radic Biol Med 2002;33(8):1115-20.

399.Ruef J, Peter K, Nordt TK, Runge MS, Kubler W, Bode C. Oxidative stress and atherosclerosis: its relationship to growth factors, thrombus formation and therapeutic approaches. Thromb Haemost 1999;82 Suppl 1:32-7.

400.Logan AC, Katzman M. Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 2005;64(3):533-8.

401.Albers DS, Beal MF. Mitochondrial dysfunction and oxidative stress in aging and neurodegenerative disease. J Neural Transm Suppl 2000;59:133-54.

402.Lee Y, Aono M, Laskowitz D, Warner DS, Pearlstein RD. Apolipoprotein E protects against oxidative stress in mixed neuronal-glial cell cultures by reducing glutamate toxicity. Neurochem Int 2004;44(2):107-18.

403. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol 1989;129(4):687-702.

404.Abate C, Ferrari-Ramondo V, Di Iorio A. Risk factors for cognitive disorders in the elderly: A review. Archives of Gerontology and Geriatrics 1998;suppl. 6:7-15.

405.Wechsler D. WAIS-R manual. Cleveland: The Psychological Corporation; 1981.

406.Peacock JM, Folsom AR, Knopman DS, Mosley TH, Goff DC, Jr., Szklo M. Dietary antioxidant intake and cognitive performance in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study investigators. Public Health Nutr 2000;3(3):337-43.

407. Russell EW. WAIS factor analysis with brain-damaged subjects using criterion measures. J Consult Clin Psychol 1972;39(1):133-9.

408.Tranel D. Neuropsychological assessment. Psychiatr Clin North Am 1992;15(2):283-99.

409.Benton AL, Eslinger PJ, Damasio AR. Normative observations on neuropsychological test performances in old age. J Clin Neuropsychol 1981;3(1):33-42.

410.Franzen MD, editor. Mutlilingual aphasia examination. Kansas City, MO: Test Corporation of America; 1986.

411.Frerichs RJ, Tuokko HA. Reliable change scores and their relation to perceived change in memory: Implications for the diagnosis of mild cognitive impairment. Arch Clin Neuropsychol 2005.

412.STATA. Statistics/Data Analysis: Release 8.2. In. Texas: Stata Corporation; 2002.

413.McCallum R. Factor Analysis, PSYC 236 coursepack: UNC Student Stores; 2004.

414.Mueller CW, Kim JO. Factor Analysis: Statistical Methods and Practical Issues. London: Sage Publications; 1978.

415.Sharma S. Applied multivariate techniques. USA: Wiley; 1996.

416.Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122(1):51-65.

417.Consumer and Food Economics Institute, editor. Agriculture handbook series. Washington DC: Agricultural Research Service; 1976-89.

418.Jain M, Cook GM, Davis FG, Grace MG, Howe GR, Miller AB. A case-control study of diet and colo-rectal cancer. Int J Cancer 1980;26(6):757-68.

419. Lyon JL, Gardner JW, West DW, Mahoney AM. Methodological issues in epidemiological studies of diet and cancer. Cancer Res 1983;43(5 Suppl):2392s-2396s.

420.Gordon T, Fisher M, Rifkind BM. Some difficulties inherent in the interpretation of dietary data from free-living populations. Am J Clin Nutr 1984;39(1):152-6.

421.Michels KB, Bingham SA, Luben R, Welch AA, Day NE. The effect of correlated measurement error in multivariate models of diet. Am J Epidemiol 2004;160(1):59-67.

422.Shahar E, Boland LL, Folsom AR, Tockman MS, McGovern PG, Eckfeldt JH. Docosahexaenoic acid and smoking-related chronic obstructive pulmonary disease. The Atherosclerosis Risk in Communities Study Investigators. Am J Respir Crit Care Med 1999;159(6):1780-5.

423.Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE. Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Am J Clin Nutr 1995;62(3):572-8.

424.Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36(5):936-42.

425.Richardson MT, Ainsworth BE, Wu HC, Jacobs DR, Jr., Leon AS. Ability of the Atherosclerosis Risk in Communities (ARIC)/Baecke Questionnaire to assess leisure-time physical activity. Int J Epidemiol 1995;24(4):685-93.

426.Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama 2001;285(19):2486-97.

427.Appels A, Hoppener P, Mulder P. A questionnaire to assess premonitory symptoms of myocardial infarction. Int J Cardiol 1987;17(1):15-24.

428. Golden SH, Williams JE, Ford DE, Yeh HC, Paton Sanford C, Nieto FJ, et al. Depressive symptoms and the risk of type 2 diabetes: the Atherosclerosis Risk in Communities study. Diabetes Care 2004;27(2):429-35.

429. Fraser GE, Butler TL, Shavlik D. Correlations between estimated and true dietary intakes: using two instrumental variables. Ann Epidemiol 2005;15(7):509-18.

430.Johansson I, Hallmans G, Wikman A, Biessy C, Riboli E, Kaaks R. Validation and calibration of food-frequency questionnaire measurements in the Northern Sweden Health and Disease cohort. Public Health Nutr 2002;5(3):487-96.

431.Fraser GE, Stram DO. Regression calibration in studies with correlated variables measured with error. Am J Epidemiol 2001;154(9):836-44.

432.Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires : the Eating at America's Table Study. Am J Epidemiol 2001;154(12):1089-99.

433.Shatenstein B, Nadon S, Godin C, Ferland G. Development and validation of a food frequency questionnaire. Can J Diet Pract Res 2005;66(2):67-75.

434.Date C, Fukui M, Yamamoto A, Wakai K, Ozeki A, Motohashi Y, et al. Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC study. J Epidemiol 2005;15 Suppl 1:S9-23.

435.Kabagambe EK, Baylin A, Allan DA, Siles X, Spiegelman D, Campos H. Application of the method of triads to evaluate the performance of food frequency questionnaires and biomarkers as indicators of long-term dietary intake. Am J Epidemiol 2001;154(12):1126-35.

436.Moilanen T, Rasanen L, Viikari J, Akerblom HK, Nikkari T. Correlation of serum fatty acid composition with dietary intake data in children and young adults. Ann Med 1992;24(1):67-70.

437.Clandinin MT, Foxwell A, Goh YK, Layne K, Jumpsen JA. Omega-3 fatty acid intake results in a relationship between the fatty acid composition of LDL cholesterol ester and LDL cholesterol content in humans. Biochim Biophys Acta 1997;1346(3):247-52.

438.Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hanninen O, Uusitupa MI. Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. Lipids 1997;32(7):697-705.

439.Zock PL, Mensink RP, Harryvan J, de Vries JH, Katan MB. Fatty acids in serum cholesteryl esters as quantitative biomarkers of dietary intake in humans. Am J Epidemiol 1997;145(12):1114-22.

440.Amiano P, Dorronsoro M, de Renobales M, Ruiz de Gordoa JC, Irigoien I. Very-long-chain omega-3 fatty acids as markers for habitual fish intake in a population consuming mainly lean fish: the EPIC cohort of Gipuzkoa. European Prospective Investigation into Cancer and Nutrition. Eur J Clin Nutr 2001;55(10):827-32.

441.Byrne B. Structural Equation Modeling with Amos: Basic Concepts, Applications, and Programming. USA; 2001.

442.Hu L, Bentler PM. Cutoff criteria in fix indexes in covariance structure analysis: conventional criteria versus new alternatives. Structural Equation Modeling 1999;6(1):1-55.

443.Bollen K. Structural equations with latent variables. New York; 1989.

444.Rosner B, Willett WC, Spiegelman D. Correction of logistic regression relative risk estimates and confidence intervals for systematic within-person measurement error. Stat Med 1989;8(9):1051-69; discussion 1071-3.

445.Carroll RJ, Stefanski LA. Approximate quasi-likelihood estimation with surrogate predictors. Journal of the American Statistical Association 1990;85:652-663.

446. Kuha J. Corrections for exposure measurement error in logistic regression models with an application to nutritional data. Stat Med 1994;13(11):1135-48.

447.Carroll RJ, Ruppert D, Stefanski LA. Measurement error in Nonlinear Models: CRC Press; 1995.

448.Holcomb JP, Jr. Regression with covariates and outcome calculated from a common set of variables measured with error: estimation using the SIMEX method. Stat Med 1999;18(21):2847-62.

449.Hardin JW, Schemiediche H, Carroll RJ. The simulation extrapolation method for fitting generalized linear models with additive measurement error. The STATA Journal 2003;4:373-385.

450.Hardin JW, Schmiediche H., Carroll RJ. The Regression Calibration Method for Fitting Generalized Linear Models with Additive Measurement Error. The STATA Journal 2003;4(1-11).

451.Budtz-Jorgensen E, Keiding N, Grandjean P, Weihe P, White RF. Consequences of exposure measurement error for confounder identification in environmental epidemiology. Stat Med 2003;22(19):3089-100.

452.Kipnis V, Subar AF, Midthune D, Freedman LS, Ballard-Barbash R, Troiano RP, et al. Structure of dietary measurement error: results of the OPEN biomarker study. Am J Epidemiol 2003;158(1):14-21; discussion 22-6.

453. Wakai K, Tamakoshi K, Date C, Fukui M, Suzuki S, Lin Y, et al. Dietary intakes of fat and fatty acids and risk of breast cancer: a prospective study in Japan. Cancer Sci 2005;96(9):590-9.

454.Terry PD, Terry JB, Rohan TE. Long-chain (n-3) fatty acid intake and risk of cancers of the breast and the prostate: recent epidemiological studies, biological mechanisms, and directions for future research. J Nutr 2004;134(12 Suppl):3412S-3420S.

455.Kaaks R, Riboli E, van Staveren W. Calibration of dietary intake measurements in prospective cohort studies. Am J Epidemiol 1995;142(5):548-56.

456. Spiegelman D, Zhao B, Kim J. Correlated errors in biased surrogates: study designs and methods for measurement error correction. Stat Med 2005;24(11):1657-82.

457.Willet WC. Nutritional epidemiology. New York: Oxford University Press; 1990.

458.Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol 1993;138(11):923-36.

459.Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Del Parigi A, Capurso S, et al. Dietary fatty acids intake: possible role in cognitive decline and dementia. Exp Gerontol 2005;40(4):257-70.

460.Maclean CH, Issa AM, Newberry SJ, Mojica WA, Morton SC, Garland RH, et al. Effects of omega-3 fatty acids on cognitive function with aging, dementia, and neurological diseases. Evid Rep Technol Assess (Summ) 2005(114):1-3.

461.Whalley LJ, Fox HC, Wahle KW, Starr JM, Deary IJ. Cognitive aging, childhood intelligence, and the use of food supplements: possible involvement of n-3 fatty acids. Am J Clin Nutr 2004;80(6):1650-7.

462.Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish Consumption and Cognitive Decline With Age in a Large Community Study. Arch Neurol 2005.

463.Kuriki K, Nagaya T, Tokudome Y, Imaeda N, Fujiwara N, Sato J, et al. Plasma concentrations of (n-3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: a cross-sectional study. J Nutr 2003;133(11):3643-50.

464.Shi J, Perry G, Smith MA, Friedland RP. Vascular abnormalities: the insidious pathogenesis of Alzheimer's disease. Neurobiol Aging 2000;21(2):357-61.

465.McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. Neurobiol Aging 2001;22(6):799-809.

466.Keli SO, Feskens EJ, Kromhout D. Fish consumption and risk of stroke. The Zutphen Study. Stroke 1994;25(2):328-32.

467.Bonaa KH, Bjerve KS, Straume B, Gram IT, Thelle D. Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromso study. N Engl J Med 1990;322(12):795-801.

468.Migliore L, Fontana I, Colognato R, Coppede F, Siciliano G, Murri L. Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol Aging 2005;26(5):587-95.