The Relationships of Dietary Fat and Blood Lipids with Brain MRI Measures

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Abstract

Objectives: This study examined the relation of dietary fat intake and blood lipid levels in a young African American and white population with subclinical MRI measures taken at a later age.

Methods: Dietary intake was assessed 3 times over 20 years and fasting blood lipids measured x times over 25 years among CARDIA participants who were 18-30 years old at baseline (n=5111). Brain MRI measures were taken at year 25 in a sub-sample (n=690). Spearman partial correlations were used to evaluate relations of dietary intake and blood lipid levels with MRI measurements; analyses were stratified by gender and adjusted for age, race, education, smoking, energy and alcohol intakes, BMI, and intracranial volume.

Results: Intakes of energy, alcohol and % kcal from total fat and saturated fat were greater in men than women. Total fat intake was not related to any brain MRI measures. However, both grams and % kcal from saturated fat were inversely related to white matter fractional anisotropy (FA) in men only (r = -0.12; p=0.03 and r=-0.14; p=0.01, respectively). Further, grams and % kcal from saturated fat were positively related to abnormal white matter tissue volume in women only (r=0.13; p=0.01 and r= 0.13; p=0.02, respectively). Omega3 fatty acids were inversely correlated to abnormal white matter (men: r=-0.11; p=0.05, women: r=-0.14; p=0.01). Additionally, omega3 fatty acids were inversely correlated with total ventricle volume in men (r=-0.11; p=0.05), but not in women.

In men there were significant inverse correlations between total cholesterol and total brain volume (r=0.12, p=0.03) . Furthermore, there were significant positive correlations between both total (r=0.11, p=0.05) and LDL-cholesterol (r=0.12, p=0.04) and total ventricle volume in men. In women there was a significant inverse correlation between triglycerides and total brain volume (r=-0.12, p=0.03) and normal gray matter (r=-0.15, p<0.01). In addition, there was also a significant inverse relationship between total cholesterol and total brain volume (r=-0.11, p=0.05) in women.

Conclusions: Given these study findings, we conclude that omega3 fatty acid intake and saturated fat may be associated with brain MRI measures in a beneficial and detrimental manner, respectively. Our studies further reveal interesting inverse relations between total and normal gray brain volumes with blood lipids in men and women. Additionally total ventricle volume was positively associated with blood lipids in men. Further research is needed to elucidate the relations of dietary fat intake and blood lipids with brain MRI measures

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A. Introduction

A.1 Specific Aims

It has been shown that dietary intake of fatty acids as well as blood lipids may alter brain status in both positive and negative ways depending on the exposure of interest. Omega 3 fatty acids and elevated HDL-cholesterol levels are correlated with improved cognitive function and a decreased risk for stroke whereas other studies show that saturated fatty acid intake, elevated plasma LDL-cholesterol, and triglycerides are positively associated with decreased cognitive function and an increased risk for stroke. There is a critical need to determine the extent of the relationships of dietary fat intake and blood lipids with markers of brain function with regard to brain function and stroke.

The objective of this study is to determine the relationships of dietary fat intake and plasma lipids--traditional risk factors for vascular disease—with brain function parameters: brain volume and fractional anisotropy (FA). Magnetic resonance imaging (MRI) measures which express these variables within specific regions of the brain as well as the whole brain are available in 791 participants at one exam in the Coronary Artery Risk Development in Young Adults (CARDIA) study, a multi-center population-based prospective study of CVD risk factor evolution in black and white men and women. Brain volume is known to elucidate the degree of appropriate brain function and activity. The FA measurements reveal the organization of axon tracts. Our central hypothesis is that high saturated fatty acid intake, total cholesterol, blood LDL-c, and triglycerides will predict the brain parameters associated with reduced brain function and brain markers of stroke, while omega-3 fatty acids and HDL-c will be inversely related. The rationale is

that traditional risk factors for CVD observed at an early age will be associated with MRI measures indicative of stroke and cognitive function at a later age. Our specific aims are to:

1) **Determine the associations of dietary intake of saturated and omega 3 fatty acids with MRI measures in the brain which indicate function and markers of stroke.**

2) **Determine the relationship between blood lipids and MRI measures in the brain which indicate function and markers of stroke.**

Our expectation is that our study will fill the large knowledge gap between traditional risk factors for CVD and the resulting state within the brain as characterized by MRI parameters. Our study population is unique given the young age and two race groups including white and African American men and women. Never before have these parameters of brain function and structure been examined relative to CVD risk factors in a younger population. The significance of this project is that identifying, early on, lifestyle and clinical factors associated with these brain markers of function and stroke will allow for the development of appropriate intervention strategies to prevent or slow down the progression of cognitive decline and the development of stroke. Our research will form the basis for future intervention studies to confirm the importance of optimal CVD risk factors for brain health and the prevention of stroke.

A.2 Background

Stroke is the third or fourth leading cause of death in the United States and causes more long-term disabilities than any other disease (Towfighi and Saver 2011, 2351-2355). Stroke is caused by a compromise of blood flow to the brain by either a blockage of blood vessels supply the brain (ischemic stroke) or blood vessel rupture (hemorrhagic stroke) (Raji et al. 2010a, 353-364). There are well-established relationships between MRI measures in the brain and the presence of stroke and cognitive dysfunction in human populations. Similarly risk factors for CVD including dietary intake of fatty acids, and blood lipids are also risk factors for stroke and cognitive dysfunction. What remains unknown, however, are the relationships between these CVD risk factors and the relevant MRI measures in the brain which likely account for subsequent ischemia, hemorrhage, and cognitive dysfunction. Thus, to prevent adverse alterations in the brain, it is of great importance, as a first step, to identify in a young population risk factors that are related to these measures in the brain which are characteristic in populations at risk for stroke and cognitive decline. The following MRI measures in the brain are associated with stroke and cognitive decline:

A.3 Normal/Abnormal Tissue Volume and Fractional Anisotropy (FA)

Reduced brain volume in non-ventricular brain tissue matter and increased axonal discontinuity, as assessed with FA measurements, are characteristic of cognitive decline (Tiberio et al. 2005, 1001-1007). FA measurements reflect the alignment and structural

integrity of fiber tracts in the brain (Basser 1995, 333-344). Thus a lower FA numeric value reflects an increased severity of tissue damage and disorganization of axonal projections (Inglese et al. 2005, 298-303; Huisman et al. 2004, 370-376). Similarly following a stroke, there is a reduction in FA due to the cellular damage from ischemia (Werring et al. 2000, 269-272). There are no published studies, that we are aware, which report the relation between CVD risk factors and FA in any human populations. Our study will assess these relationships.

The relative mass of abnormal volume of regions of interest reflects the presence of ischemic tissue or an area associated with brain atrophy, reduced CBF, gait disorders, and cognitive impairment (DeCarli et al. 1995a, 2077-2084). Increased abnormal white matter intensity is associated with the risk for stroke, concurrent silent stroke, and cognitive decline in populations of healthy adults (DeCarli et al. 1995b, 2077-2084; Longstreth et al. 1996, 1274-1282). Reduced brain volume in the gray and white matter is associated with various brain diseases such as schizophrenia, attention-deficit-disorders, and autism (Tiberio et al. 2005, 1001-1007). Whereas increased ventricle volume is associated with cognitive decline and dementia (Haxby et al. 1992, 2029-2029) and with other cardiovascular risk factors including diabetes in the ARIC study cohort (Knopman et al. 2005, 876-881). Blood LDL-c is a risk factor for impairment related to brain volume analyses at single time points in elderly populations (Raji et al. 2010b, 353-364). However, our study will assess these relationships in a young population where longitudinal analysis may be applied.

A.4 Dietary Fatty Acid Intake and Brain Measures

It is known that fatty acid intake has marked roles with regard to brain function. Saturated fat intake has a pronounced negative impact on the function of the brain in addition to its well-known role in the promotion of heart disease. Saturated fat intake has been shown to increase risk for dementia whereas omega-3 fatty acids have a protective effect on cognitive function in the elderly (Del Parigi et al. 2006, 1-19). Additionally a diet high in saturated fat is associated with increased rates of cognitive decline over 6 years in a biracial community population (Morris et al. 2004, 1573-1579). Thus it is likely that decreases in brain volume and axonal density may be present within the brain as a result of a high intake of saturated-fatty acids. Evidence suggests that dietary intake of saturated fatty acids results in alterations in plasma phospholipid composition and subsequent stroke risk. In humans, plasma phospholipid saturated fatty acids are associated with an increased risk for stroke (Wiberg et al. 2006, 2898-2903). However a meta-analysis in 8 studies reveals that the dynamics of this relationship are unclear (Scarborough et al. 2010, 458).

Omega-3 fatty acids are known to be required for the development and function of both the brain and the retina (Neuringer, Anderson, and Connor 1988, 517-541). Omega-3 fatty acids compose the membranes of brain cells as well as participate in the regulation of blood flow within the cerebrum (Bourre 2004). Omega-3 fatty acids are also necessary for synapse development and thus affect the density of axonal projections (McGahon et al. 1999, 305-314). Omega-3 fatty acid supplementation has been associated with a

reversal of cerebral atrophy in schizophrenia and Huntington's Disease and a reduction in ventricular volume in treatment-resistant depression (Puri et al. 2001, 560; Puri et al. 2000, 57; Puri et al. 2002, 123; Logan 2003, 410-425). Omega-3 fatty acid supplementation results in neuroprotection from ischemic events in neonates and likely occurs through its anti-inflammatory action (Zhang et al. 2010, 2341-2347). Similarly an increased intake of omega-3 fatty acid-containing fish is associated with a decreased risk for ischemic stroke in men and women (Larsson and Orsini 2011; Larsson, Virtamo, and Wolk 2011, 487-493).

We will examine the relations of both dietary intake of saturated and omega3 fatty acids with brain normal and abnormal volume, and fractional anisotropy.

A.5 Blood Lipids and Brain Measures

Little research has been done to determine the associations between blood cholesterol and triglycerides with MRI measures of brain function and stroke. In particular we are interested in the association between blood total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride levels and MRI measures in the brain taken subsequently. In one study high levels of LDL-cholesterol were associated with a low gray matter volume (Whalley et al. 2003, 173-176). Blood LDL-cholesterol is also associated with an increasing number of lesions in the brains of patients with multiple sclerosis (Giubilei et al. 2002, 109-112). Similarly blood levels of oxidized

LDL-cholesterol are associated with ischemic lesion detected by diffusion weighted MRI imaging and predicts the size of brain infarctions (Uno et al. 2005, 94-102).

With regard to cognitive function, elevated blood LDL and decreased blood HDL-cholesterol are risk factors for vascular dementia (Reitz et al. 2004, 705). High total cholesterol levels are associated with memory dysfunction and cognitive decline (Desmond et al. 1993, 162), (Yaffe et al. 2002, 378). We predict that blood LDL-cholesterol and triglyceride levels will be positively associated with reduced brain volume and axonal organization assessed with FA measurements. Blood HDL-cholesterol is positively associated with cognitive performance in a population of centenarians and is expected to be positively associated with measures of cognitive performance (Atzmon et al. 2002, M712). We will examine the relation of blood lipids with normal/abnormal brain volume and fractional anisotropy.

Through our available data set we have access to dietary intake and blood lipid variables as well as the described recently acquired MRI parameters. We will be able to determine correlations between prior dietary fatty acid intake and blood lipids and the following MRI parameters. Determination of these associations will prove very useful in preventative measures with regard to stroke and brain function. We expect to establish important relationships that will affect the health of millions nationwide.

A.6 Definitions

Adverse/Negative brain function (with regard to cognitive function and stroke) is

characterized by:

- decreased gray matter volume: marker of cognitive function
- increased white matter volume: marker of cognitive function and stroke
- increased ventricular volume: marker of cognitive function
- decreased FA: marker of cognitive function and stroke

A.7 Hypothesis

We hypothesize that dietary intake of saturated fatty acids will be negatively related to brain function while omega 3 fatty acids will be positively related to function. We hypothesize that total cholesterol, LDL-c and triglycerides will be negatively related to brain function whereas HDL-c will be positively related to brain function in a young to middle-aged adult population.

A.8 Hypothetical Mechanisms

Our hypothesis that dietary intake of saturated fatty acids will be negatively associated with brain function may occur through several potential mechanisms. One mechanism involves changes in the composition of phospholipids in the plasma membrane. Dietary intake of saturated fat is associated with alteration in phophatidylcholine profile in neuronal membranes indicating that membrane permeability and integrity may be affected (Greenwood and Winocur 1996, 451).

Activation of astroglial cells by physiological processes results in subsequent fluctuations in brain tissue oxygenation and potentially cognitive function. Specifically, cross-talk between neurons and glial cells resulting in an influx of calcium into glial cells accounts for fluctuations in BOLD signal detected by fMRI (DiNuzzo et al. 2011, 3010-3018; Brenneman and Rutledge 1979, 295-304). Additionally saturated fatty acids result in an alteration of dopamine and norepinephrine transport in the cerebral cortex of rats via changes in membrane fluidity (Brenneman and Rutledge 1979, 295-304).

A potential mechanism to account for the effect of saturated fat intake and elevated blood lipids on brain function involves the upregulation of inflammatory processes. High fat diets are shown to increase the formation of reactive oxygen species and NADPH oxidate-mediated oxidative stress (Zhang et al. 2005, 318-325). High-fat diets also increase inflammatory prostaglandin 2, COS-2, and NKFB translocation in the cerebral cortex of rats (Zhang et al. 2005, 318-325). Physiological processes that affect cortical dynamics via astrocytes in mammals may involve metabolic responses to dietary challenges such as dietary saturated fatty acids intake or metabolic imbalance due to elevated blood LDL-c and triglycerides (Cunningham et al. 2006, 5597-5601). Additionally direct effects of these stressors on inflammatory processes could result in calcium influx and subsequent activation of glial cells resulting in deterioration of brain processes. Blood flow acts directly on astrocytes in the brain and vasoactive compounds present in the blood have marked changes on astrocyte and subsequent brain activity (Rossi 2006, 159-161).

Omega-3 fatty acids affect brain function through several mechanisms involving effects on neurons signaling and physical processes such as membrane fluidity and bilayer thickness (Horrocks and Farooqui 2004, 361-372). The importance of these mechanisms is supported by the fact that deficiencies of docosahexanoic acid are associated with decreased neuron signaling, gene expression, and inflammation (Horrocks and Farooqui 2004, 361-372). Thus it may be reasonably proposed that omega-3 fatty acid intakes result in decreased inflammation and improved brain function and integrity through, for example, the expression of appropriate genes required for neuronal survival and growth.

A.9 Research Design and Methods

Overview

We have available through the Coronary Artery Risk Development in Young Adults (CARDIA) data from participants of this observational study about the etiology of cardiovascular disease. At year 0, 2, 5, 7, 10, 15, and 20, dietary intake measurements of fatty acids intake an/ord blood lipids were obtained from the participants. At year 25 a brain MRI analysis was performed on these participants. The MRI variables for which we have data include brain volume and FA. Data for these variables is reported as individual data values within voxels or within particular regions of the brain (RIO: Regions of Interest) as well as for the whole brain. Our main approach in our studies is to assess how

prior dietary intake of nutrients such as fatty acids and blood lipids predicts these MRI parameters at year 25.

Study Participants

The data for our proposed study comes from the Coronary Artery Risk Development in Young Adults (CARDIA) cohort of white and black men and women in the United States. Data are acquired from 791 participants between the ages of 18 and 30 years old at baseline (1985-1986). Exclusion criteria include unusually high (> 8000 kcal for men and >6000 kcal for women) or low (<800 for men and <600 for women) caloric intake at year 0 or 7, the presence of pregnancy or lactation in women at year 0 or 7, participants who had elevated blood pressure or diabetes at baseline, and participants with non-fasting blood samples. Blood pressure measurements taken during pregnancy were treated as missing. Additional exclusion criteria were implemented for MRI scan participants. Participation for MRI scanning was reduced due to disinterest, absence, claustrophobia, ineligibility, scheduling conflict, MRI contraindication, clinical MRI alert levels of 3 or 4, or scanning technical difficulties. Following the implementation of exclusion criteria, the total sample in these analyses was n=724; including black men (n = 137), black women (n = 160), white men (n = 207), and white women (n = 220). All participants gave written consent, and the institutional review boards of the participating field centers gave approval for the study.

Data Acquisition

For the brain MRI study, the coordinating and clinical centers are located in Birmingham, AL, Minneapolis, MN, and Oakland, CA. The participants have been followed up for assessment of clinical factors at years 0, 2, 5, 7, 10, 15, 20, and 25 years from baseline. Magnetic Resonance Imaging (MRI) data at year 25 were acquired from these participants.

Clinical Assessment of Blood Lipid Parameters

For analysis of blood lipid parameters, subjects fasted for >8 hrs and were asked to avoid smoking and physical activity for two hours prior to the CARDIA exam. Blood samples were collected and stored in vials at -70 degrees C. The clinical parameter of importance for our proposed study is the plasma levels of triglycerides, a risk factor for coronary heart disease (Wilson et al. 1998, 1837-1847).

CARDIA Diet History Assessment

Dietary intake at years 0, 7, and 20 was determined based on interviewer-administered food intake questionnaires. The standard CARDIA diet questionnaire queried food intake, frequency of consumption and portion size. Condiments and type of fat used in preparation or added at the table were also queried. This was important for our assessment of dietary fatty acid intake. Daily nutrient intake was calculated using nutrient data from the National Nutrient Database for Standard Reference (NNDS) which

incorporated the macronutrient and micronutrient content of 18,000 foods and 7,000 brand products. This database is known for its comprehensiveness with regard to the vast number of food variations and the components of measurements.

The reliability and comparative validity of the CARDIA dietary history questionnaire was assessed in participants. Seven telephone-assessed 24-hour dietary intake recalls were administered and compared with the standard CARDIA estimation of daily dietary intake (Liu et al. 1994, 15). No significant differences were observed between reported nutrient intakes assessed by both these methods in white participants. In black participants there was less consistency. Overall this study verified the reasonability of this CARDIA dietary questionnaire in accurately predicting nutrient intake. The dietary parameters which are of interest to our proposed study include the following:

Omega-3 and saturated fatty acid intake. Based on reported intake of various food types, we are able to calculate the intake of both omega-3 fatty acids and saturated fatty acids.

M*agnetic Resonance Imaging Data*

At year 25, participants underwent an MRI analysis. An initial testing scan was performed for review and approval by the University of Pennsylvania Medical Center. Study scans were performed at the three field centers and acquired data was sent to the MRI reading centers in the University of Pennsylvania where a DICOM image transfer system was used for analysis of data. All field center personnel involved were appropriately trained for MRI scan acquisition. All participants were required to sign consent forms and were educated on the scanning procedures prior to the hour-long

session. Field centers were required to rigorously follow scanning protocol and maintain participant comfort throughout the scanning procedures.

Scanning pulse sequences were performed in the order of first to last in the following list: 3 plane Gradient echo localizer for positioning, Sagittal 3D T1-weighted sequence for entire brain coverage, Sagittal 3D FLAIR images from matching slice positions in Series 2, Sagittal 3D T2-weighted images matching slice positions in Series 2, Axial ASL (Arterial Spin Labeling) perfusion sequence, Axial ASL (Arterial Spin Labeling) calibration sequence, Axial DTI (Diffusion Tensor Imaging) sequence, Axial BOLD (Blood Oxygen Level Dependent) FMRI sequence (resting state), Axial BOLD (Blood Oxygen Level Dependent) FMRI sequence (16 second breath-hold).Results were sent to the coordinating center for data storage and transfer to coordinating universities (University of Minnesota in our case).

The DICOM analysis calculated various parameters within brain regions (voxel-derived) and in larger portions of the brain including the whole brain. The database of MRI data includes values for many brain regions of interest including the whole brain, gray matter, white matter, ventricles, cerebellum, the major gyri, and many other smaller areas. The following parameters are included in our analysis:

Brain Volume: Brain volume calculations measure the volume in cubic centimeters (cm^3) within each region of interest. Brain volumes are categorized as either normal or

abnormal volume. We are interested in the volumes of our chosen regions of interest with respect to our exposures.

FA: The mean FA numeric measurements are acquired through diffusion tensor MRI Thus we are able to characterize the specific organization of neuronal circuits as related to our exposures.

Regions of Interest

We will assess each of the described MRI parameters in the following regions of interest:

Whole brain: Analysis of the whole brain will give rise to nonspecific general measures of cognitive function. This region of interest is of particular interest with regard to volume, CBF, anisotropy, and the diffusion coefficient. Whole brain measurements have been used for analysis and prognosis of a variety of cognitive diseases (Abell et al. 1999, 1647; Alavi et al. 1993, 1681).

Gray Matter: Gray matter consists of the major central nervous system components: the neuron cell bodies along with attached dendrites and axons, the capillaries, and the glial cells which play a supportive role to the surrounding neurons. Decreased gray matter volume or MRI hypointensity are associated with cognitive impairment (Brass et al.

2006, 437-444). Reduced gray matter volume and damage to this area is characteristic of neurological diseases such as multiple sclerosis (Geurts and Barkhof 2008, 841-851).

White Matter: White matter is composed of glial cells and myelin sheaths that cover tracts of axons. Lesions in the white matter are associated with cognitive dysfunction due to the role of white matter in conveying signal between difference regions in the brain (de Groot et al. 2000, 145-151).

Ventricle: The ventricles are large spaces present in the brain which contain circulating cerebrospinal fluid. The ventricular system is composed of the third ventricle, the right and left lateral ventricles, and fourth ventricle. The total volume of the ventricles is detected by the MRI measures and will be used for our analysis. Increased ventricular volume accompanies reduced brain volume and aging indicating reduced function and cognitive performance (Scahill et al. 2003, 989; DeCarli et al. 1995c, 2077-2084; Johnstone et al. 1976, 924-926).

Statistical Analysis

The program PC-SAS software (version 8; SAS Institute, Cary, NC) was used for all data analyses. For all exposures, variables were treated as continuous variables averaged over 20 years (years 0, 2, 5, 7, 15, or 20). Distributions were examined for normality and skewed distributions normalized where appropriate. Descriptive statistics for continuous

variables were presented as means (SD) or (SE) when adjusted for confounding factors and frequencies (%) for categorical variables by sex group. Spearman correlations evaluated the relations of dietary fatty acid intake and plasma lipids with brain MRI measures by gender. These models were adjusted for several confounding factors, including age, race, field center, education, smoking, family history of heart disease/stroke, BMI, OC use, and other potential confounding factors.

The employed statistical models incorporated several potential confounding factors. Family history of heart disease and stroke is available through the CARDIA data set and considered as a potential confounder in the analysis. This adjustment accounted for some genetic variables and hereditary propensities for these diseases and the associated disease risk (Neufeld and Goldbourt 1983, 943-954; Alberts 1991, 276-280). Age was adjusted for in the models to remove any age related progression of MRI components (Raz et al. 2005, 1676-1689). Data were stratified by gender to compensate, for example, for neuroprotective effects of estrogen and progesterone (ROOF and HALL 2000, 367-388). Physical activity was also adjusted for to account for proposed benefits on cognitive function (56). Additionally, we also accounted for race, field center, and socioeconomical status.

B. The Relation between Dietary Intake of Fat and Brain MRI Measures

B.1 Introduction

Epidemiologic studies have shown that dietary intake influences cognitive function and risk of stroke assessed with brain MRI measures such as brain volume and fractional anisotropy (Tangney et al. 2011, 1276-1282; Gardener et al. 2012, 251). Although few studies have examined the relations of dietary fat intake and subclinical measures of brain function, several studies have shown inverse relations of dietary fat intake with cognitive function and stroke. Cross-sectional studies in middle-aged adults have shown that high total fat and saturated fat intakes were associated with greater risk for cognitive impairment assessed by an extensive battery of cognitive tests which measured memory and motor function (Kalmijn et al. 2004, 275-280; Ortega et al. 1997, 803-809). Prospective studies in elderly adults have shown positive associations between dietary saturated fat and risk for cognitive decline (Morris et al. 2004, 1573-1579). Conversely, saturated fat intake has been inconsistently related to the development of stroke (Micha and Mozaffarian 2010, 893-905). A meta-analysis of 21 prospective studies with 5-23 years of follow up found that saturated fat consumption assessed by dietary recalls or food frequency questionnaires was not related to risk of stroke (0.81, 95 % CI: 0.62-1.05) (Siri-Tarino et al. 2010, 535-546; Corvol et al. 2003, 669). The inconsistent results of the relations between saturated fat and cognitive function and stroke may be due to interplay of oxidative stress and neurochemicals involved in the energy homeostasis of brain cells

(Wu, Ying, and Gomez-Pinilla 2004, 1699-1707) In addition, the differences in measurement or assessment of stroke and cognitive function in these studies, such as the extensive battery of motor, memory, attention, and other behavioral measurements for cognitive function or the assessment of stroke, such as using the number of strokes or size of ischemic lesions as the outcome, may also account for the differential study findings ((DeCarli et al. 1995b, 2077-2084; Bowman et al. 2012, 241-249).

Omega3 fatty acid intake has been positively related to both cognitive function and inversely with stroke outcome in animal studies as well as in cross-sectional studies among elderly adults (Zhang et al. 2005, 318-325; Kalmijn et al. 2004, 275-280). An analysis of 15 prospective studies with 4-30 years of follow-up in middle-aged to elderly adults from several countries showed that the consumption of 3 servings of fish per week, a common source of omega3 fatty acid intake, is related to a lower risk of stroke (Larsson and Orsini 2011). Further, omega3 fatty acids have also been associated with a decreased risk of cognitive impairment in elderly adults and that high fish consumption was associated with a slower progression of cognitive decline over 5 years in middle-aged adults (van Gelder et al. 2007, 1142-1147; Kalmijn et al. 2004, 275-280). One study reported lower incidence of subclinical infarcts and better white matter grade with greater fish intake in older adults (Virtanen et al. 2008, 439-446). In another cross-sectional study conducted among older adults, a dietary intake pattern rich in omega3 fatty acids was beneficially related to cognitive function and white matter hyperintensity (Bowman et al. 2012, 241-249). Dietary fatty acid intake in young adults relative to brain MRI measures obtained at middle age has not been studied.

Using data from the Coronary Artery Risk Development in Young Adults (CARDIA) study, we examined the associations of dietary fat intake over 20 years with structural and functional brain MRI measures at a later age. We hypothesize that average saturated fat intake over 20 years will be inversely related to total brain volume, normal gray matter volume, normal white matter volume, and white matter FA and positively related to ventricular and abnormal white matter volume assessed 25 years post-baseline. Further, we expect positive relations of omega3 fatty acid intake with total brain volume, gray matter volume, normal white matter volume, and fractional anisotropy and an inverse relation with ventricular and abnormal white matter volume.

B.2 Methods

The Institutional Review Board at each institution approved the study protocols and all study participants signed informed consent forms.

Study Population

The CARDIA study is a prospective study of the development and progression of CVD risk factors in 5,115 young African American and Caucasian adults aged 18-30 years at baseline (1985-87) recruited at 4 field centers located in Birmingham, AL, Chicago, IL, Minneapolis, MN, and Oakland, CA. Eligibility criteria for a brain MRI scan included interest in participation, not claustrophobic, not pregnant, no presence of

MRI-contraindication, weighing less than 350 pounds, and under 50 inches for waist and hip circumferences. Of the 2018 people approached from the Birmingham, Minneapolis, and Oakland field centers, 791 men and women volunteered to have a brain MRI scan at year 25. After exclusion criteria were applied, 719 individuals were scanned and 710 and 701 individuals had valid structural and functional measurements, respectively, after MRI exclusions due to technical and participant difficulties.

Inclusion criteria for these analyses are valid MRI measurements at year 25 and dietary intake assessments for at least two exams among years 0, 7 and 20. Observations were excluded for unusually high (>8000 kcal for men and >6000 kcal for women) or low (<800 for men and <600 for women) energy intake at year 0, 7, or 20 (n=4). Individuals with missing baseline BMI (n=5) and smoking status (n=6) were excluded. The total sample included in the analysis was n=690 for those with volume measurements and n=680 for those with fractional anisotropy measurements.

Diet Assessment

See section A.9, CARDIA Diet History Assessment (page 12)

Magnetic Resonance Imaging

At year 25, eligible participants from the three field centers Birmingham, AL, Minneapolis, MN, and Oakland, CA underwent an MRI scan by trained personnel after pilot testing and approval by the University of Pennsylvania Medical Center. Scanning pulse sequences were performed in the order of first to last as follows: 3 plane

Gradient echo localizer for positioning, Sagittal 3D T1-weighted sequence for entire brain coverage, Sagittal 3D Fluid Attenuated Inversion Recovery (FLAIR) images from matching slice positions in Series 2, Sagittal 3D T2-weighted images matching slice positions in Series 2, Axial Arterial Spin Labeling (ASL) perfusion sequence, Axial ASL calibration sequence, and Axial Diffusion Tensor Imaging (DTI) sequence.

The analysis of MRI images was performed using an automatic algorithm which removed extra-cerebral tissue and segmented the brain parenchyma into gray matter, white matter, and cerebrospinal fluid. The scanned image was transferred to regions of interest (ROIs) from the Jakob Atlas (Shen and Davatzikos 2002, 1421-1439). White Matter Lesion Segmentation was used to segment small vessel ischemic disease within data sets in order to classify each voxel as either normal or abnormal tissue (Zacharaki et al. 2008, 620-627). White matter integrity was evaluated by fitting a tensor to each DTI volume and used to calculate fractional anisotropy.

For this analysis, the brain regions of interest include whole brain, gray matter, white matter, and ventricles. The brain MRI outcomes include total brain volume, normal gray and white matter volume, abnormal white matter volume, and total ventricles, which were all expressed in cm^3; and white matter FA. Total ventricles were defined as the total volume of the third and both lateral ventricles.

Statistical Analysis

The SAS software (version 8; SAS Institute, Cary, NC) was used for all analysis. The distributions of brain MRI measures were checked for normality. Dietary intakes at years 0, 7, and 20 were averaged and included in the models as a continuous variable. Descriptive statistics for continuous variables were presented as means (SD) and categorical variables presented as frequencies (%). Because differential results have been observed for cognitive decline in men and women (22), effect modification of gender was evaluated on the relation between dietary fat intake and brain MRI measures(p for interaction <0.05 was found for abnormal white matter, total ventricle volume, normal gray matter, normal brain volume, and normal white volume). Thus, Spearman partial correlations by gender evaluated the relations between dietary fat intakes and brain MRI measures, adjusting for age, race, year 25 education, year 25 smoking, average (year 0,15, and 20) energy intake, average (year 0,15, and 20) alcohol intake, change in BMI between year 0 and 25, y0 BMI, and total intracranial volume. P-values less than or equal to 0.05 were deemed as statistically significant.

B.3 Results

The average age at the time of the MRI scan (year 25) was 50.2 years for males and 50.4 years for females (Table 1). Women reported significantly more years of education than men; while the proportion of current smokers and alcohol drinkers was similar for both men and women. At baseline, men reported greater intake for energy (kcal), alcohol

(g/day), and % kcal from total and saturated fat, while women reported greater % kcal from carbohydrates (Table 1). Although BMI was similar between men and women, systolic and diastolic blood pressures were greater among men than women.

Ventricle volume and intracranial volume were significantly greater in men than in women (Table 2). Abnormal white volume was significantly greater in women than in men, although the volumes were quite small for both sexes and right-skewed.

Spearman partial correlation coefficients evaluated the relations between the 20-year average of dietary fat intake and brain MRI measures (Table 3). Total fat intake was not related to any of the brain MRI measures in men or women. Saturated fat (% and gm) was correlated with abnormal white matter in women only. In both men and women, there were significant inverse correlations between omega3 fatty acid intake (% and mg) and abnormal white matter volume. In men, but not women, saturated fatty acids (% and g) were inversely correlated with white matter FA and omega3 fatty acid intake (%) was inversely correlated with total ventricle volume.

B.4 Discussion

We observed in both men and women significant inverse correlations between omega3 fatty acids and abnormal white matter volume. Although, saturated fat was positively related to abnormal white matter volume in women, this relation was not

significant in men. Further, we observed in men an inverse correlation between saturated fat and white matter FA. Finally, omega3 fatty acids were inversely related to ventricle volume in men, but not women. Though, little research has been conducted in this area, our study results support the findings of studies that have examined the relations of dietary fat intake with cognitive function (or decline) and stroke. These results support the theory that a healthier dietary fat intake including intake of omega3 fatty acids are inversely associated with white matter abnormalities in men and women respectively.

Omega3 fatty acids play an important role in the development and maintenance of brain function (21), supporting our observation that omega3 fatty acid intake over 20 years was beneficially associated with brain MRI measures in middle-aged adults. Omega3 fatty acids are required for development and function of both the brain and the retina (Neuringer, Anderson, and Connor 1988, 517-541), are involved in the regulation of blood flow within the cerebrum (Bourre 2004), as well as being necessary for synapse development and thus affect the density of axonal projections (McGahon et al. 1999, 305-314). Supplemental omega3 fatty acids have been associated with a reversal of cerebral atrophy in schizophrenia and Huntington's Disease as well as in the reduction of ventricular volume in treatment-resistant depression (Puri et al. 2000, 57; Puri et al. 2002, 123; Logan 2003, 410-425). Omega3 fatty acid supplementation resulted in neuroprotection from ischemic events in neonates and likely occurs through its anti-inflammatory action (Zhang et al. 2010, 2341-2347). Similarly an increased intake of omega3 fatty acid-containing fish has been associated with a decreased risk for ischemic

stroke in men and women (Larsson and Orsini 2011; Larsson, Virtamo, and Wolk 2011, 487-493).

Omega3 fatty acids affect brain function through several mechanisms involving effects on neuron signaling and physical processes such as membrane fluidity and bilayer thickness (Horrocks and Farooqui 2004, 361-372). The importance of these mechanisms is supported by evidence that deficiencies of docosahexanoic acid are associated with decreased neuron signaling, gene expression, and inflammation (Horrocks and Farooqui 2004, 361-372). Thus it is reasonable that omega3 fatty acid intakes may result in decreased inflammation and improved brain function and integrity through, for example, the expression of appropriate genes required for neuronal survival and growth (Horrocks and Farooqui 2004, 361-372).

As we expected, saturated fat intake was inversely associated with white matter integrity in men. Saturated fat intake has had a pronounced negative impact on the function of the brain in addition to its well-known role in the promotion of heart disease. Saturated fat has been related to increased risk for dementia (Del Parigi et al. 2006, 1-19). Additionally, a diet high in saturated fat has been associated with increased risk of cognitive decline over 6 years in a biracial community population (Morris et al. 2004, 1573-1579).

The effect of dietary saturated fat intake on brain MRI measures may occur through several potential mechanisms. One mechanism involves changes in the composition of phospholipids in the plasma membrane. Dietary intake of saturated fat is associated with alteration in phosphatidylcholine profile in neuronal membranes indicating that

membrane permeability and integrity may be affected (Greenwood and Winocur 1996, 451). Another potential mechanism to account for the effect of saturated fat intake on brain function involves the upregulation of inflammatory processes. High fat diets are shown to increase the formation of reactive oxygen species and NADPH-mediated oxidative stress (Zhang et al. 2005, 318-325). Further, high-fat diets also increase inflammatory prostaglandin 2, COS-2, and NKFB translocation in the cerebral cortex of rats (Zhang et al. 2005, 318-325). Physiological processes that affect cortical dynamics via astrocytes in mammals may involve metabolic responses to dietary challenges such as dietary saturated fatty acids (Cunningham et al. 2006, 5597-5601). Direct effects of these stressors on inflammatory processes could result in calcium influx and subsequent activation of glial cells resulting in deterioration of brain processes. Blood flow acts directly on astrocytes in the brain and vasoactive compounds present in the blood have marked changes on astrocyte and subsequent brain activity (Rossi 2006, 159-161).

The differences in the relations involving saturated fat and omega3 fatty acid intake between men and women could be explained by the structural and connective gender disparities in the brain. Studies show that there are structural differences as well as differences in cerebral blood flow between men and women which may affect the mechanistic pathway for the effects of diet on the brain (Schlaepfer et al. 1995, 129-135). Similarly differences exist in the connectivity between brain hemispheres and the effects of brain connectivity on cognition between men and women (Davatzikos and Resnick 1998, 635-640). This may account for our findings in men, but not in women, of an inverse correlation between saturated fat and white matter FA. Additionally the presence

of the estrogenic effect on omega3 fatty acid storage in women may account for the differences in the effects on this nutrient on the brain (Giltay et al. 2004, 1167-1174). It was of particular importance that we examined the correlations separately for men and women to clarify these gender specific effects. The absence of any significant associations between gray matter and the MRI measures could be due to inadequate sample size. Additionally, the effect of diet on the volume of the gray matter may involve nutrient-nutrient or gene-nutrient interactions.

Strengths and limitations

One limitation of our study was the use of self-reported dietary intake. However, the CARDIA diet history questionnaire was validated (McDonald et al. 1991, 1104). Further, averaging dietary intake across years 0, 7, and 20 increased the precision within reported individual dietary intake. Additionally, assessment of diet using the CARDIA diet history captured brand names of fat products used in food preparation and added at the table which increased the validity of fatty acid intake. Knowing the type of fatty acids in the food product facilitates correct classification of type of fat intake, and therefore, reduces the measurement error of dietary fat intake. A common limitation with traditional brain MRI analysis is measurement error. However, in this study high resolution, 3T, 3D isotropic imaging was performed and statistically sophisticated voxel-based analysis was used which improved the accuracy and precision in measurement and quality control samples were performed. Another limitation is that MRI measures were not taken at any time points prior to year 25. Thus, conclusions about the earlier relations between dietary fat and brain MRI remain unknown. A major strength was the

prospective study design of our study examining usual dietary fat intake over 20 years relative to brain MRI measures at year 25 in a biracial population of adult men and women.

B.5 Conclusion

Our study sheds light on important associations between earlier dietary fat intake and the subsequent brain MRI measures that are indicative of overall brain health. In addition, our findings support the general recommendation to limit intake of saturated fat for maintaining brain health in addition to the widely discussed cardiovascular health benefits. Future studies are warranted to examine these relations in larger populations and over a longer period of time.

C. The Relation between Blood Lipid Levels and Brain MRI Measures

C.1 Introduction

MRI measures such as brain volume and fractional anisotropy, a marker of axonal organization and directional congruency, are subclinical markers of cognitive function and stroke risk (Tiberio et al. 2005, 1001-1007; Longstreth et al. 1996, 1274-1282) Little research has been done to determine the associations between blood cholesterol and

triglycerides with MRI measures of brain function and stroke markers. In particular we are interested in the association between blood low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride levels and MRI measures in the brain taken 25 years later.

A cross-sectional study in 44 ischemic patients showed that blood levels of oxidized LDL-cholesterol are associated with ischemic lesion detected by diffusion weighted MRI imaging and predicts the size of brain infarction (Uno et al. 2005, 94-102). A cross-sectional study in 139 centarians showed that blood HDL-cholesterol is positively associated with cognitive performance and is expected to be positively associated with measures of cognitive performance (Atzmon et al. 2002, M712). In a study of 89 non-demented old people high levels of total and LDL-cholesterol were associated with a low gray matter volume (Whalley et al. 2003, 173-176). In a population of 18 multiple sclerosis patients assessed over a 6 month period, blood total and LDL-cholesterol was associated with an increasing number of lesions in the brain (Giubilei et al. 2002, 109-112).

With regard to cognitive function, cross-sectional and prospective studies involving 4316 Medicare recipients over 65 years of age from Manhattan, NY showed that elevated blood LDL and decreased blood HDL-cholesterol are risk factors for vascular dementia (Reitz et al. 2004, 705). High total cholesterol levels are associated with memory dysfunction and cognitive decline in a longitudinal study with 249 community volunteers around 70 years of age (Desmond et al. 1993, 162). A 4 year follow-up longitudinal study with 1037 postmenopausal women with cardiovascular heart disease found that LDL

cholesterol with associated with cognitive impairment as assessed with a mini mental state examination (Yaffe et al. 2002, 378).

Few studies have examined the influence of blood lipids on subclinical MRI measures of the brain, especially in younger adults. Therefore, we examined the associations of early exposure to blood lipid levels (age 18-30 years at baseline) with structural and functional brain MRI measures at a later age (43-55 years). We examined the relation of blood lipids with normal/abnormal brain volume and fractional anisotropy. We predict that blood LDL-cholesterol and triglyceride levels will be positively associated with reduced brain volume and axonal organization assessed with fractional anisoptropy measurements. We also predict that HDL-cholesterol will be associated with greater brain volume and axonal organization.

C.2 Methods

All participants of our study signed informed consent forms prior to the study and the Institutional Review Board at each of the three involved institution approved of the study protocols.

Study Population

See Section A.9, page 11 for description of the population

Inclusion criteria for these analyses are valid MRI measurements at year 25 and valid

blood lipid measurements. Additionally a minority of individuals scanned in the Chicago center were excluded (n=5). After this exclusion the total study sample was 710. Following missing MRI data exclusions and missing confounding variable data (n=20), the total sample was 690 for volume measurements and 680 for fractional anisotropy measurements.

Clinical Assessment of Blood Lipid Parameters

Subjects fasted for >8 hrs and were asked to avoid smoking and physical activity for two hours prior to the CARDIA exam for the analysis of blood lipid parameters. The blood samples were stored at -70 degrees Celcius. The clinical parameter of importance for our proposed study is the plasma levels of total cholesterol and triglycerides, risk factors for coronary heart disease (Wilson et al. 1998, 1837-1847); however, LDL-c and HDL-c were also examined.

M*agnetic Resonance Imaging*

See section A.9 (page 13-14) for description of MRI

Statistical Analysis

The SAS software (version 8; SAS Institute, Cary, NC) was used for all analysis. The distribution for abnormal matter was skewed, however we employed spearman

correlations in our analysis to normalize the distribution. Because studies of cognitive decline showed differences by gender group, data were stratified (Gur et al. 1991, 2845). Blood lipid levels at years 0, 2, 5, 7, 10, 15, 20, and 25 were averaged and included in the models as a continuous variable.

Descriptive statistics for continuous variables were presented as means (SD) or (SE).. Spearman correlation analysis was used to evaluate the relations between blood lipid levels over 25 years and brain MRI measures at year 25 stratified by gender, adjusting for age, race, year 25 education, year 25 smoking, % kcal from saturated fat (average of years 0, 7, and 20), change in BMI between year 0 and 25, baseline BMI, alcohol intake (average of years 0, 7, and 20) and intracerebral volume. P-values less than or equal to 0.05 were deemed as significant.

C.3 Results

As previously described in Table 2, ventricle volume and intracranial volume were significantly greater in men than in women. Abnormal white volume was significantly greater in women than in men. White matter FA was greater in women than men. Total cholesterol was similar between men and women; however, triglycerides and LDL-cholesterol were higher in men than women, while HDL-cholesterol levels were higher in women (Table 4).

Table 5 shows the Spearman partial correlation coefficients for the relations between

the 25-year average blood lipids and the MRI measures. In men there were significant inverse relations between total cholesterol and total brain volume. In men there were significant positive correlations for both total cholesterol and LDL-cholesterol with total ventricle volume. In women there was a significant inverse correlations for total cholesterol and triglycerides with total brain volume and an inverse correlation between triglycerides and normal gray matter.

C.4 Discussion

Our study findings support our hypothesis that total cholesterol is inversely associated with total brain volume in men and women and that triglycerides are inversely associated with total brain and normal gray volumes in women. In men total and LDL cholesterol was positively associated with total ventricle volume. It is possible the beneficial effects of HDL cholesterol on brain function reported in prior studies that examined older populations suggesting the relationship may be strengthened with increasing age (Atzmon et al. 2002, M712; Reitz et al. 2004, 705).

Blood lipids may influence the MRI parameters through effects on inflammation, brain function, and neuronal integrity. For example, synaptogensis and the effect of cholesterol homeostasis on the neuronal proteome and neuron degeneration may be affected (Goritz, Mauch, and Pfrieger 2005, 190-201; Tsaousidou et al. 2008, 510-515; Wang et al. 2008, 1606-1614). It is possible that decreases in brain volume and axonal density may be present within the brain as a result of elevated cholesterol.

The correlation between blood lipid levels and brain MRI measures may be accounted for through several potential mechanisms. One mechanism involves an inflammation-mediated damage of the blood brain barrier. Blood lipids have an established and marked role on the upregulation of local inflammation (Abela 2010, 156-164). For example, a study in rabbits showed that dietary cholesterol doubled the levels of beta-amyloid in the hippocampal cortex which may be related to the observed appearance of beta-amyloid immunoreactivity in the neuropil. The authors concluded that cholesterol-mediated vascular inflammation can lead to the breaking of the blood-brain barrier integrity and produce biochemical alterations that result in the formation of the beta-amyloid protein (Sparks et al. 2000, 335-344). It is expected that the disruption in the blood brain barrier may cause other alterations in the brain and subsequent inflammation. Additionally direct effects of these stressors on inflammatory processes could result in calcium influx and subsequent activation of glial cells resulting in deterioration of brain processes. Changes in blood flow that occur as a result of blood lipid levels could affect the activity of astrocytes. Blood flow acts directly on astrocytes in the brain and affects subsequent brain activity (Rossi 2006, 159-161; Rossi 2006, 159-161). Activation of astroglial cells by physiological processes results in subsequent fluctuations in brain tissue oxygenation and potentially cognitive function. Specifically, cross-talk between neurons and glial cells resulting in an influx of calcium into glial cells accounts for fluctuations in BOLD signal detected by fMRI (Rossi 2006, 159-161). The differences in the relationships involving saturated blood lipids between men and women could be explained by the structural and connective gender disparities in the brain. Studies show that there are structural and

vascular differences between men and women which may affect the mechanistic pathway for the effects of blood lipids on the brain (Schlaepfer et al. 1995, 129-135). Similarly differences exist in the connectivity between brain hemispheres and the effects of brain connectivity on cognition between men and women (Davatzikos and Resnick 1998, 635-640).

To elucidate the biological impact of the previously observed significant relationships further research is required. Specifically, the impact of the potential effects of cardiovascular disease risk factors on MRI measures upon the biological processes involved in the development and progression of cognitive malfunction and stroke requires studies examining protein expression within cells, and other biological assays within the brain, for example.

Strengths and limitations

A common limitation with traditional brain MRI analysis is measurement error. However, this study utilized a sophisticated voxel-based analysis was used which improved the accuracy and precision in measurement. A major strength was the prospective study design of our study examining blood lipid levels over 25 years relative to brain MRI measures at year 25 in a biracial population of adults. The measurement of brain MRI parameters at a time subsequent to when blood lipids were measured allows for a prospective analysis of the observed correlations. In contrast to other studies, blood lipids in our population were assessed for over 25 years beginning in young adulthood.

C.5 Conclusion

Our results suggest that early exposure to blood lipids affect the status of the brain as assessed by MRI measures taken at a later age. Thus, the idea that the blood lipid levels in younger populations are important for the maintenance of proper cognitive function and the prevention of cerebrovascular and neurodegenerative diseases is supported. The previously observed benefits of HDL-cholesterol on the brain may be manifested later on in the life-span than the middle-age of the individuals used for the brain MRI scans in our study. It is also possible that a larger sample size is necessary to see the effect. Future studies may look at these relationships in larger populations and over a longer period of time. For example, it would be important to see how blood lipid levels in younger or older populations affect the status of the brain of the same population at an elderly age. It is likely that levels of blood lipids in the healthy range are important at all stages of the life cycle for brain health.

D. Future Directions

In light of the relationships observed in these studies between dietary fat intake and blood lipids with subsequent brain MRI measures, there are several future directions which would be appropriate to support the theories and mechanisms which are responsible for a potential causative effect. A combination of cell, animal, and human studies, both short and long-term, would elucidate the mechanisms and direction of

effect. The following are ideal future studies:

- Brain cell culture studies examining the effect of blood lipids on intracellular processes
- Animal studies which examine long term effects of dietary fat (both omega and saturated) on brain MRI measures
- Animal studies which examine short term effect of dietary fat overload on inflammatory markers and brain MRI measures
- Human prospective studies similar to those done here with a larger n size
- Human intervention studies examining short term effects of dietary fat on brain MRI measures
- Human intervention/cohort studies examining long-term effects of dietary fat on brain MRI measures
- Human clinical studies examining treatment of cognitive decline and/or stroke propensity with omega-3 dietary fat

References

Abela, G. S. 2010. "Cholesterol Crystals Piercing the Arterial Plaque and Intima Trigger Local and Systemic Inflammation." *Journal of Clinical Lipidology* 4 (3): 156-164.

Abell, F., M. Krams, J. Ashburner, R. Passingham, K. Friston, R. Frackowiak, F. Happé, C. Frith, and U. Frith. 1999. "The Neuroanatomy of Autism: A Voxel-Based Whole Brain Analysis of Structural Scans." *Neuroreport* 10 (8): 1647.

Alavi, A., A. B. Newberg, E. Souder, and J. A. Berlin. 1993. "Quantitative Analysis of PET and MRI Data in Normal Aging and Alzheimer's Disease: Atrophy Weighted Total Brain Metabolism and Absolute Whole Brain Metabolism as Reliable Discriminators." *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine* 34 (10): 1681.

Alberts, MJ. 1991. "Genetic Aspects of Cerebrovascular Disease." *Stroke* 22 (2): 276-280.

Atzmon, G., I. Gabriely, W. Greiner, D. Davidson, C. Schechter, and N. Barzilai. 2002. "Plasma HDL Levels Highly Correlate with Cognitive Function in Exceptional Longevity." *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 57 (11): M712.

Basser, P. J. 1995. "Inferring Microstructural Features and the Physiological State of Tissues from Diffusion□weighted Images." *NMR in Biomedicine* 8 (7): 333-344.

Bourre, JM. 2004. "Roles of Unsaturated Fatty Acids (especially Omega-3 Fatty Acids) in the Brain at various Ages and during Ageing." *J Nutr Health Aging* 8 (3).

Bowman, GL, LC Silbert, D. Howieson, HH Dodge, MG Traber, B. Frei, JA Kaye, J. Shannon, and JF Quinn. 2012. "Nutrient Biomarker Patterns, Cognitive Function, and MRI Measures of Brain Aging." *Neurology* 78 (4): 241-249.

Brass, SD, R. H. B. Benedict, B. Weinstock-Guttman, F. Munschauer, and R. Bakshi. 2006. "Cognitive Impairment is Associated with Subcortical Magnetic Resonance Imaging Grey Matter T2 Hypointensity in Multiple Sclerosis." *Multiple Sclerosis* 12 (4): 437-444.

Brenneman, D. E. and C. O. Rutledge. 1979. "Alteration of Catecholamine Uptake in Cerebral Cortex from Rats Fed a Saturated Fat Diet." *Brain Research* 179 (2): 295-304.

Corvol, J. C., A. Bouzamondo, M. Sirol, J. S. Hulot, P. Sanchez, and P. Lechat. 2003. "Differential Effects of Lipid-Lowering Therapies on Stroke Prevention: A Meta-Analysis of Randomized Trials." *Archives of Internal Medicine* 163 (6): 669.

Cunningham, MO, DD Pervouchine, C. Racca, NJ Kopell, CH Davies, RSG Jones, RD Traub, and MA Whittington. 2006. "Neuronal Metabolism Governs Cortical Network Response State." *Proceedings of the National Academy of Sciences* 103 (14): 5597-5601.

Davatzikos, C. and SM Resnick. 1998. "Sex Differences in Anatomic Measures of Interhemispheric Connectivity: Correlations with Cognition in Women but Not Men." *Cerebral Cortex* 8 (7): 635-640.

de Groot, J. C., M. Oudkerk, J. Gijn, A. Hofman, J. Jolles, and MM Breteler. 2000. "Cerebral White Matter Lesions and Cognitive Function: The Rotterdam Scan Study." *Annals of Neurology* 47 (2): 145-151.

DeCarli, C., DGM Murphy, M. Tranh, CL Grady, J. V. Haxby, JA Gillette, JA Salerno, A. Gonzales-Aviles, B. Honvitz, and SI Rapoport. 1995a. "The Effect of White Matter Hyperintensity Volume on Brain Structure, Cognitive Performance, and Cerebral Metabolism of Glucose in 51 Healthy Adults." *Neurology* 45 (11): 2077-2084.

———. 1995b. "The Effect of White Matter Hyperintensity Volume on Brain Structure, Cognitive Performance, and Cerebral Metabolism of Glucose in 51 Healthy Adults." *Neurology* 45 (11): 2077-2084.

———. 1995c. "The Effect of White Matter Hyperintensity Volume on Brain Structure, Cognitive Performance, and Cerebral Metabolism of Glucose in 51 Healthy Adults." *Neurology* 45 (11): 2077-2084.

Del Parigi, A., F. Panza, C. Capurso, and V. Solfrizzi. 2006. "Nutritional Factors, Cognitive Decline, and Dementia." *Brain Research Bulletin* 69 (1): 1-19.

Desmond, D. W., T. K. Tatemichi, M. Paik, and Y. Stern. 1993. "Risk Factors for Cerebrovascular Disease as Correlates of Cognitive Function in a Stroke-Free Cohort." *Archives of Neurology* 50 (2): 162.

DiNuzzo, M., T. Gili, B. Maraviglia, and F. Giove. 2011. "Modeling the Contribution of Neuron-Astrocyte Cross Talk to Slow Blood Oxygenation Level-Dependent Signal Oscillations." *Journal of Neurophysiology* 106 (6): 3010-3018.

Gardener, Hannah, Nikolaos Scarmeas, Yian Gu, Bernadette Boden-Albala, Mitchell SV Elkind, Ralph L. Sacco, Charles DeCarli, and Clinton B. Wright. 2012.

"Mediterranean Diet and White Matter Hyperintensity Volume in the Northern Manhattan Study." *Archives of Neurology* 69 (2): 251.

Geurts, J. J. G. and F. Barkhof. 2008. "Grey Matter Pathology in Multiple Sclerosis." *The Lancet Neurology* 7 (9): 841-851.

Giltay, E. J., L. J. G. Gooren, A. W. F. T. Toorians, M. B. Katan, and P. L. Zock. 2004. "Docosahexaenoic Acid Concentrations are Higher in Women than in Men because of Estrogenic Effects." *The American Journal of Clinical Nutrition* 80 (5): 1167-1174.

Giubilei, F., G. Antonini, S. Di Legge, MP Sormani, P. Pantano, R. Antonini, M. Sepe□Monti, F. Caramia, and C. Pozzilli. 2002. "Blood Cholesterol and MRI Activity in First Clinical Episode Suggestive of Multiple Sclerosis." *Acta Neurologica Scandinavica* 106 (2): 109-112.

Goritz, C., D. H. Mauch, and F. W. Pfrieger. 2005. "Multiple Mechanisms Mediate Cholesterol-Induced Synaptogenesis in a CNS Neuron." *Molecular and Cellular Neuroscience* 29 (2): 190-201.

Greenwood, C. E. and G. Winocur. 1996. "Cognitive Impairment in Rats Fed High-Fat Diets: A Specific Effect of Saturated Fatty-Acid Intake." *Behavioral Neuroscience* 110 (3): 451.

Gur, R. C., P. D. Mozley, S. M. Resnick, G. L. Gottlieb, M. Kohn, R. Zimmerman, G. Herman, S. Atlas, R. Grossman, and D. Berretta. 1991. "Gender Differences in Age Effect on Brain Atrophy Measured by Magnetic Resonance Imaging." *Proceedings of the National Academy of Sciences* 88 (7): 2845.

Haxby, JV, JA Gillette, D. Teichberg, SI Rapoport, and MB Schapiro. 1992. "Longitudinal Changes in Lateral Ventricular Volume in Datients with Dementia of the Alzheimer Type." *Neurology* 42 (10): 2029-2029.

Horrocks, L. A. and A. A. Farooqui. 2004. "Docosahexaenoic Acid in the Diet: Its Importance in Maintenance and Restoration of Neural Membrane Function." *Prostaglandins, Leukotrienes and Essential Fatty Acids* 70 (4): 361-372.

Huisman, T. A. G. M., L. H. Schwamm, P. W. Schaefer, W. J. Koroshetz, N. Shetty-Alva, Y. Ozsunar, O. Wu, and A. G. Sorensen. 2004. "Diffusion Tensor Imaging as Potential Biomarker of White Matter Injury in Diffuse Axonal Injury." *American Journal of Neuroradiology* 25 (3): 370-376.

Inglese, M., S. Makani, G. Johnson, B. A. Cohen, J. A. Silver, O. Gonen, and R. I. Grossman. 2005. "Diffuse Axonal Injury in Mild Traumatic Brain Injury: A Diffusion Tensor Imaging Study." *Journal of Neurosurgery* 103 (2): 298-303.

Johnstone, E. C., CD Frith, TJ Crow, J. Husband, and L. Kreel. 1976. "Cerebral Ventricular Size and Cognitive Impairment in Chronic Schizophrenia." *The Lancet* 308 (7992): 924-926.

Kalmijn, S., MPJ Van Boxtel, M. Ocke, WMM Verschuren, D. Kromhout, and LJ Launer. 2004. "Dietary Intake of Fatty Acids and Fish in Relation to Cognitive Performance at Middle Age." *Neurology* 62 (2): 275-280.

Knopman, D. S., T. H. Mosley, D. J. Catellier, and A. R. Sharrett. 2005. "Cardiovascular Risk Factors and Cerebral Atrophy in a Middle-Aged Cohort." *Neurology* 65 (6): 876-881.

Larsson, S. C. and N. Orsini. 2011. "Fish Consumption and the Risk of Stroke." *Stroke*.

Larsson, S. C., J. Virtamo, and A. Wolk. 2011. "Fish Consumption and Risk of Stroke in Swedish Women." *The American Journal of Clinical Nutrition* 93 (3): 487-493.

Liu, K., M. Slattery, D. Jacobs Jr, G. Cutter, A. McDonald, L. Van Horn, JE Hilner, B. Caan, C. Bragg, and A. Dyer. 1994. "A Study of the Reliability and Comparative Validity of the Cardia Dietary History." *Ethnicity & Disease* 4 (1): 15.

Logan, A. C. 2003. "Neurobehavioral Aspects of Omega-3 Fatty Acids: Possible Mechanisms and Therapeutic Value in Major Depression." *Alternative Medicine Review* 8 (4): 410-425.

Longstreth, WT, T. A. Manolio, A. Arnold, G. L. Burke, N. Bryan, C. A. Jungreis, P. L. Enright, D. O'Leary, and L. Fried. 1996. "Clinical Correlates of White Matter Findings on Cranial Magnetic Resonance Imaging of 3301 Elderly People: The Cardiovascular Health Study." *Stroke* 27 (8): 1274-1282.

McDonald, A., L. Van Horn, M. Slattery, J. Hilner, C. Bragg, B. Caan, D. Jacobs Jr, K. Liu, H. Hubert, and N. Gernhofer. 1991. "The CARDIA Dietary History: Development, Implementation, and Evaluation." *Journal of the American Dietetic Association* 91 (9): 1104.

McGahon, BM, DSD Martin, DF Horrobin, and MA Lynch. 1999. "Age-Related Changes in Synaptic Function: Analysis of the Effect of Dietary Supplementation with [Omega]-3 Fatty Acids." *Neuroscience* 94 (1): 305-314.

Micha, R. and D. Mozaffarian. 2010. "Saturated Fat and Cardiometabolic Risk Factors, Coronary Heart Disease, Stroke, and Diabetes: A Fresh Look at the Evidence." *Lipids* 45 (10): 893-905.

Morris, MC, DA Evans, JL Bienias, CC Tangney, and RS Wilson. 2004. "Dietary Fat Intake and 6-Year Cognitive Change in an Older Biracial Community Population." *Neurology* 62 (9): 1573-1579.

Neufeld, HN and U. Goldbourt. 1983. "Coronary Heart Disease: Genetic Aspects." *Circulation* 67 (5): 943-954.

Neuringer, M., G. J. Anderson, and W. E. Connor. 1988. "The Essentiality of N-3 Fatty Acids for the Development and Function of the Retina and Brain." *Annual Review of Nutrition* 8 (1): 517-541.

Ortega, R. M., A. M. Requejo, P. Andres, A. M. Lopez-Sobaler, M. E. Quintas, M. R. Redondo, B. Navia, and T. Rivas. 1997. "Dietary Intake and Cognitive Function in a Group of Elderly People." *The American Journal of Clinical Nutrition* 66 (4): 803-809.

Puri, B. K., G. M. Bydder, S. J. Counsell, B. J. Corridan, A. J. Richardson, J. V. Hajnal, C. Appel, H. M. McKee, K. S. Vaddadi, and D. F. Horrobin. 2002. "MRI and Neuropsychological Improvement in Huntington Disease Following Ethyl-EPA Treatment." *Neuroreport* 13 (1): 123.

Puri, BK, SJ Counsell, G. Hamilton, AJ Richardson, and DF Horrobin. 2001. "Eicosapentaenoic Acid in Treatment-Resistant Depression Associated with Symptom Remission, Structural Brain Changes and Reduced Neuronal Phospholipid Turnover." *International Journal of Clinical Practice* 55 (8): 560.

Puri, BK, AJ Richardson, DF Horrobin, T. Easton, N. Saeed, A. Oatridge, JV Hajnal, and GM Bydder. 2000. "Eicosapentaenoic Acid Treatment in Schizophrenia Associated with Symptom Remission, Normalisation of Blood Fatty Acids, Reduced Neuronal Membrane Phospholipid Turnover and Structural Brain Changes." *International Journal of Clinical Practice* 54 (1): 57.

Raji, C. A., A. J. Ho, N. N. Parikshak, J. T. Becker, O. L. Lopez, L. H. Kuller, X. Hua, A. D. Leow, A. W. Toga, and P. M. Thompson. 2010a. "Brain Structure and Obesity." *Human Brain Mapping* 31 (3): 353-364.

———. 2010b. "Brain Structure and Obesity." *Human Brain Mapping* 31 (3): 353-364.

Raz, N., U. Lindenberger, K. M. Rodrigue, K. M. Kennedy, D. Head, A. Williamson, C. Dahle, D. Gerstorf, and J. D. Acker. 2005. "Regional Brain Changes in Aging

Healthy Adults: General Trends, Individual Differences and Modifiers." *Cerebral Cortex* 15 (11): 1676-1689.

Reitz, C., M. X. Tang, J. Luchsinger, and R. Mayeux. 2004. "Relation of Plasma Lipids to Alzheimer Disease and Vascular Dementia." *Archives of Neurology* 61 (5): 705.

ROOF, R. L. and E. D. HALL. 2000. "Gender Differences in Acute CNS Trauma and Stroke: Neuroprotective Effects of Estrogen and Progesterone." *Journal of Neurotrauma* 17 (5): 367-388.

Rossi, D. J. 2006. "Another BOLD Role for Astrocytes: Coupling Blood Flow to Neural Activity." *Nature Neuroscience* 9 (2): 159-161.

Scahill, R. I., C. Frost, R. Jenkins, J. L. Whitwell, M. N. Rossor, and N. C. Fox. 2003. "A Longitudinal Study of Brain Volume Changes in Normal Aging using Serial Registered Magnetic Resonance Imaging." *Archives of Neurology* 60 (7): 989.

Scarborough, P., M. Rayner, I. van Dis, and K. Norum. 2010. "Meta-Analysis of Effect of Saturated Fat Intake on Cardiovascular Disease: Overadjustment Obscures True Associations." *The American Journal of Clinical Nutrition* 92 (2): 458.

Schlaepfer, T. E., G. J. Harris, A. Y. Tien, L. Peng, S. Lee, and G. D. Pearlson. 1995. "Structural Differences in the Cerebral Cortex of Healthy Female and Male Subjects: A Magnetic Resonance Imaging Study." *Psychiatry Research: Neuroimaging* 61 (3): 129-135.

Shen, Dinggang and Christos Davatzikos. 2002. "HAMMER: Hierarchical Attribute Matching Mechanism for Elastic Registration." *Medical Imaging, IEEE Transactions On* 21 (11): 1421-1439.

Siri-Tarino, P. W., Q. Sun, F. B. Hu, and R. M. Krauss. 2010. "Meta-Analysis of Prospective Cohort Studies Evaluating the Association of Saturated Fat with Cardiovascular Disease." *The American Journal of Clinical Nutrition* 91 (3): 535-546.

Sparks, D. L., Y. U. M. I. N. KUO, A. Roher, T. Martin, and R. J. Lukas. 2000. "Alterations of Alzheimer's Disease in the Cholesterol-fed Rabbit, Including Vascular Inflammation: Preliminary Observations." *Annals of the New York Academy of Sciences* 903 (1): 335-344.

Tangney, CC, NT Aggarwal, H. Li, RS Wilson, C. DeCarli, DA Evans, and MC Morris. 2011. "Vitamin B12, Cognition, and Brain MRI Measures." *Neurology* 77 (13): 1276-1282.

Tiberio, M., DT Chard, DR Altmann, G. Davies, CM Griffin, W. Rashid, J. Sastre-Garriga, AJ Thompson, and DH Miller. 2005. "Gray and White Matter Volume Changes in Early RRMS." *Neurology* 64 (6): 1001-1007.

Towfighi, A. and J. L. Saver. 2011. "Stroke Declines from Third to Fourth Leading Cause of Death in the United States." *Stroke* 42 (8): 2351-2355.

Tsaousidou, M. K., K. Ouahchi, T. T. Warner, Y. Yang, M. A. Simpson, N. G. Laing, P. A. Wilkinson, R. E. Madrid, H. Patel, and F. Hentati. 2008. "Sequence Alterations within CYP7B1 Implicate Defective Cholesterol Homeostasis in Motor-Neuron Degeneration." *The American Journal of Human Genetics* 82 (2): 510-515.

Uno, M., M. Harada, O. Takimoto, K. Kitazato, A. Suzue, K. Yoneda, N. Morita, H. Itabe, and S. Nagahiro. 2005. "Elevation of Plasma Oxidized LDL in Acute Stroke Patients is Associated with Ischemic Lesions Depicted by DWI and Predictive of Infarct Enlargement." *Neurological Research* 27 (1): 94-102.

van Gelder, B. M., M. Tijhuis, S. Kalmijn, and D. Kromhout. 2007. "Fish Consumption, N–3 Fatty Acids, and Subsequent 5-Y Cognitive Decline in Elderly Men: The Zutphen Elderly Study." *The American Journal of Clinical Nutrition* 85 (4): 1142-1147.

Virtanen, J. K., D. S. Siscovick, W. T. Longstreth, L. H. Kuller, and D. Mozaffarian. 2008. "Fish Consumption and Risk of Subclinical Brain Abnormalities on MRI in Older Adults." *Neurology* 71 (6): 439-446.

Wang, Y., S. Muneton, J. Sjövall, J. N. Jovanovic, and W. J. Griffiths. 2008. "The Effect of 24S-Hydroxycholesterol on Cholesterol Homeostasis in Neurons: Quantitative Changes to the Cortical Neuron Proteome." *Journal of Proteome Research* 7 (4): 1606-1614.

Werring, D. J., A. T. Toosy, C. A. Clark, G. J. M. Parker, G. J. Barker, D. H. Miller, and A. J. Thompson. 2000. "Diffusion Tensor Imaging can Detect and Quantify Corticospinal Tract Degeneration After Stroke." *Journal of Neurology, Neurosurgery & Psychiatry* 69 (2): 269-272.

Whalley, LJ, RT Staff, AD Murray, SJ Duthie, AR Collins, HA Lemmon, JM Starr, and IJ Deary. 2003. "Plasma Vitamin C, Cholesterol and Homocysteine are Associated with Grey Matter Volume Determined by MRI in Non-Demented Old People." *Neuroscience Letters* 341 (3): 173-176.

Wiberg, B., J. Sundström, J. Árnlöv, A. Terént, B. Vessby, B. Zethelius, and L. Lind. 2006. "Metabolic Risk Factors for Stroke and Transient Ischemic Attacks in Middle-Aged Men." *Stroke* 37 (12): 2898-2903.

Wilson, P. W. F., R. B. D'Agostino, D. Levy, A. M. Belanger, H. Silbershatz, and W. B. Kannel. 1998. "Prediction of Coronary Heart Disease using Risk Factor Categories." *Circulation* 97 (18): 1837-1847.

Wu, A., Z. Ying, and F. Gomez-Pinilla. 2004. "The Interplay between Oxidative Stress and Brain-derived Neurotrophic Factor Modulates the Outcome of a Saturated Fat Diet on Synaptic Plasticity and Cognition." *European Journal of Neuroscience* 19 (7): 1699-1707.

Yaffe, K., E. Barrett-Connor, F. Lin, and D. Grady. 2002. "Serum Lipoprotein Levels, Statin use, and Cognitive Function in Older Women." *Archives of Neurology* 59 (3): 378.

Zacharaki, Evangelia, Stathis Kanterakis, R. Bryan, and Christos Davatzikos. 2008. "Measuring Brain Lesion Progression with a Supervised Tissue Classification System." *Medical Image Computing and Computer-Assisted Intervention–MICCAI 2008*: 620-627.

Zhang, W., X. Hu, W. Yang, Y. Gao, and J. Chen. 2010. "Omega-3 Polyunsaturated Fatty Acid Supplementation Confers Long-Term Neuroprotection Against Neonatal Hypoxic–Ischemic Brain Injury through Anti-Inflammatory Actions." *Stroke* 41 (10): 2341-2347.

Zhang, X., F. Dong, J. Ren, M. J. Driscoll, and B. Culver. 2005. "High Dietary Fat Induces NADPH Oxidase-Associated Oxidative Stress and Inflammation in Rat Cerebral Cortex." *Experimental Neurology* 191 (2): 318-325.

Appendix 1: Tables

Table 1. Mean (SE) or frequency (%) of year 25 demographic and clinical characteristics and average dietary intake (years 0, 7, 20) among African American and Caucasian men and women, CARDIA (n=690)

Characteristics	Men (n=326)	Women (n=364)	p-value
Age, years	50.2 (0.19)	50.4 (0.18)	0.54
Race, % African American	38.2 (2.65)	42.5 (2.51)	0.24
Education, years	14.7 (0.14)	15.1 (0.13)	0.01
Current smoker, %	17.1 (2.05)	15.7 (1.94)	0.61
Current drinker, %	18.5 (2.25)	23.5 (2.12)	0.19
Daily Dietary Intake			
Energy, kcal	3321 (57)	2174 (53)	<0.001
Total fat, %kcal	36.8 (0.28)	35.8 (0.27)	0.007
PUFA, %kcal	7.3 (0.10)	7.3 (0.10)	0.99
Saturated fat, %kcal	12.9 (0.14)	12.5 (0.13)	0.03
Omega3, %kcal	0.046 (0.003)	0.051 (0.003)	0.29
Carbohydrate, %kcal	46.6 (0.36)	49.0 (0.34)	<0.001
Protein, %kcal	14.7 (0.12)	14.8 (0.12)	0.62
Alcohol , g	16.3 (0.87)	7.4 (0.82)	<0.001
Clinical characteristics			
Baseline Body mass index , (kg/m^2)	23.8 (0.24)	23.9 (0.22)	0.56
Yr 25 Systolic blood pressure, mmHG	121 (0.78)	116 (0.74)	<0.001
Yr 25 Diastolic blood pressure, mmHG	75(0.57)	72 (0.54)	0.008

*Adjusted for age and race

Table 2. Average year 25 brain MRI measures* among African American and Caucasian men and women, CARDIA (n=690)

Brain MRI Measures*	Males (n=326)	Females (n=364)	p-value
Total Brain Volume (cm^3)	978.90 (1.79)	984.08 (1.67)	0.06
Normal Gray Matter (cm^3)	514.84 (1.35)	518.49 (1.27)	0.08
Normal White Matter (cm^3)	464.06 (1.50)	465.59 (1.41)	0.50
Abnormal White Volume (cm3) **	0.20	0.30	<0.001
Total Ventricle Volume (cm3)	22.84 (0.60)	20.78 (0.56)	0.02
White Matter Fractional Anisotropy†	0.304 (0.001)	0.308 (0.001)	0.08
Intracranial Volume‡	1287 (5.60)	1138 (5.30)	<0.001

*Adjusted for age, race, and intracranial volume (ICV), except ICV;
**Abnormal white volume is reported as median and assessed with a Wilcoxon median test
†For white matter fractional anisotropy, n=324 for men and n=356 for women.
‡ICV was adjusted for age and race.

Table 3. Spearman correlation coefficients* between average (years 0, 7, 20) dietary intake and year 25 brain MRI measures among African American and Caucasian men and women, CARDIA (n=690)

Men (n=326)						
Dietary fat intake	**Normal total brain (cm3)**	**Normal gray matter (cm3)**	**Normal white matter (cm3)**	**Abnormal white matter (cm3)**	**Total ventricle volume (cm3)**	**White matter fractional anisotropy****
Total Fat (%)	0.05 P=0.39	-0.03 P=0.60	0.07 P=0.21	0.08 P=0.17	-0.07 P=0.24	-0.04 P=0.44
Total Fat (g)	0.04 P=0.49	0.01 P=0.89	0.03 P=0.66	0.09 0.12	-0.07 P=0.24	-0.04 P=0.53
Saturated Fat (%)	0.02 P=0.79	-0.01 P =0.91	0.02 P=0.72	0.07 P=0.19	-0.07 P=0.22	-0.14 P=0.01
Saturated Fat (g)	<-0.01 P=0.97	-0.01 P=0.89	<-0.01 P=0.98	0.05 P=0.37	-0.07 P=0.19	-0.12 P=0.03
Omega3 Fat (%)	-0.03 P=0.60	-0.06 P=0.29	0.05 P=0.41	-0.11 P=0.05	-0.11 P=0.05	0.09 P=0.13
Omega3 Fat (mg)	=-.02 P=0.68	-0.05 P=0.36	0.05 P=0.42	-0.14 P=0.01	-0.10 P=0.08	0.10 P=0.09
Women (n=364)						
Total Fat (%)	0.05 P=0.38	0.01 P=0.79	0.04 P=0.51	0.09 P=0.08	0.02 P=0.72	0.05 P=0.36
Total Fat (g)	0.03 P=0.64	0.01 P=0.83	0.01 P=0.81	0.06 P=0.24	0.03 P=0.61	0.04 P=0.43
Saturated Fat (%)	-0.01 P=0.82	-0.004 P=0.93	0.01 P=0.91	0.13 P=0.02	0.07 P=0.19	-0.05 P=0.38
Saturated Fat (g)	-0.01 P=0.83	-0.002 P=0.97	<-0.01 P>0.99	0.13 P=0.01	0.08 P=0.15	-0.07 P=0.18
Omega3 Fat (%)	-0.02 P=0.71	0.02 P=0.67	-0.03 P=0.61	-0.14 P=0.01	-0.01 P=0.80	0.07 P=0.20
Omega3 Fat (mg)	-0.02 P=0.78	0.02 P=0.65	-0.02. p=0.70	-0.14 P=0.01	-0.02 P=0.79	0.06 P=0.24

*Adjusted for age, race, education, y25 smoking, y0 BMI, change in BMI between y0 and y25, energy and alcohol intake, and intracranial volume (volume measures only).
**For white matter fractional anisotropy, n=324 for men and n=356 for women.

Table 4. Mean* (SE) fasting plasma lipids over 25 years among African American and Caucasian men and women, CARDIA (n=690)

Characteristics	Males (n =326)	Females (n =364)	p-value
Total Cholesterol, mg/dL	184.28 (1.59)	181.94 (1.50)	0.28
Triglycerides, mg/dL	107.95 (3.30)	79.95 (3.12)	<0.001
LDL Cholesterol, mg/dL	113.87 (1.48)	108.01 (1.40)	0.004
HDL Cholesterol, mg/dL	49.37 (0.68)	57.81 (0.64)	0.008

*Adjusted for age and race

Table 5. Spearman correlation coefficients* between average fasting plasma lipids over 25 years and year 25 brain MRI measures among African American and Caucasian men and women, CARDIA (n=690)

Men (N=326)						
Fasting plasma lipids	**Total Brain Volume (cm3)**	**Normal gray matter (cm3)**	**Normal white matter (cm3)**	**Abnormal White Matter (cm3)**	**Total Ventricle (cm3)**	**White** Matter Fractional Anisotropy**
Total Cholesterol, mg/dL	-0.12 P=0.03	-0.05 P=0.38	-0.10 P=0.07	-0.07 P=0.24	0.11 P=0.05	-0.005 P=0.93
Triglycerides (mg/dL)	-0.04 P=0.48	0.04 P=0.44	-0.10 P=0.08	-0.003 P=0.96	0.04 P=0.44	0.02 P=0.71
LDL Cholesterol (mg/dL)	-0.07 P=0.20	-0.02 P=0.76	-0.07 P=0.20	-0.07 P=0.23	0.12 P=0.04	0.01 P=0.80
HDL Cholesterol (mg/dL)	-0.04 P=0.53	-0.09 P=0.11	0.04 P=0.49	-0.02 P=0.71	-0.01 P=0.77	-0.03 P=0.55
Women (N=364)						
	Total Brain Volume (cm3)	**Normal gray matter (cm3)**	**Normal white matter (cm3)**	**Abnormal White Matter (cm3)**	**Total Ventricle (cm3)**	**White** Matter Fractional Anisotropy**
Total Cholesterol, mg/dL	-0.11 P=0.04	-0.06 P=0.23	-0.06 P=0.29	0.005 P=0.93	0.06 P=0.28	-0.03 P=0.60
Triglycerides (mg/dL)	-0.12 P=0.03	-0.15 P<0.01	-0.03 P=0.55	-0.04 P=0.47	0.08 P=0.12	-0.08 P=0.16
LDL Cholesterol (mg/dL)	-0.09 P=0.11	-0.03 P=0.53	-0.05 P=0.40	-0.01 P=0.82	0.05 P=0.33	-0.03 P=0.57
HDL Cholesterol (mg/dL)	-0.02 P=0.75	-0.01 P=0.79	0.02 P=0.76	0.03 P=0.60	-0.06 P=0.30	0.06 P=0.30

*Adjusted for age, race, education, year 25 smoking, change in BMI between years 0 and 5, year 0 BMI, average saturated fat%, and alcohol intake, and intracranial volume
**For fractional anisotropy measurements, n=324 for men and n=356 for women