THE IMPACT OF DIETARY OMEGA-3 FATTY ACIDS AND OMEGA-3 TO OMEGA-6 FATTY ACID RATIO ON COGNITIVE DECLINE IN THE HEALTHY AGING IN NEIGHBORHOODS OF DIVERSITY ACROSS THE LIFESPAN (HANDLS) STUDY SAMPLE

by

Constance Gerassimakis

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LIST OF ABBREVIATIONS

ALA, alpha-linolenic acid (18:3n-3);

ARA, arachidonic acid (20:4n-6);

Attention, Brief test of Attention total correct;

BMI, body mass index;

BVRTot, Benton Visual Retention test total errors;

CES-D, Center for Epidemiologic Studies-Depression test

CIND, cognitive impairment/no dementia;

Clock command total score;

CVLtca, California Verbal Learning test total correct List A immediate recall;

CVLfrl, California Verbal Learning test # correct List A long-delay free recall;

DPA, docosapentaenoic acid (22:5n-3);

DHA, docosahexaenoic acid (22:6n-3);

Digit span Fwd, Wechsler Adult Intelligence Scale- Revised Digit Span Forward total score;

Digit span Bck, Wechsler Adult Intelligence Scale- Revised Digit Span Backward total score;

E, energy;

EPA, eicosapentaenoic acid (20:5n-3);

FADS, fatty acid desaturase;

Fluency Word, Animal Fluency total words;

HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Lifespan;

LA, linoleic acid (18:2n-6);

MCI, mild cognitive impairment;

MEND, multimodality pilot program to enhance neurodegeneration;

MMStot, Mini-Mental State Exam total score;

n-3PUFA, omega-3 polyunsaturated fatty acids;

n-3HUFA, omega-3 highly unsaturated fatty acids;

n-6PUFA, omega-6 polyunsaturated fatty acids;

TrailsAtest Sec, Trail Making Test A test seconds;

TrailsBtest Sec, Trail Making Test B test seconds;

WRAT, The Wide Range Achievement Test- Revision 3

ABSTRACT

Background. Growing evidence suggests an association between decreased dietary intake of omega-3 fatty acids and increased risk for cognitive impairment and cognitive decline. Since the metabolic pathways of omega-3 (n-3) and omega-6 (n-6) fatty acids are mutually competitive, with chiefly antagonistic physiological effects, the ratio of n-3 to n-6 fatty acids may be a better indicator of essential fatty acid status than absolute levels.

Objective. The primary aim of this study was to evaluate the relationship of absolute n-3 fatty acid and relative n-3 to n-6 fatty acid intakes with cognitive decline in an urban biracial population of ages 30-64 years.

Methods. Our study is a prospective longitudinal examination of the relationship of baseline absolute n-3 fatty acid and relative n-3/n-6 dietary intakes with cognitive change between Wave 1 and Wave 3 measured by eleven neuropsychological tests over multiple domains. This study is a secondary analysis of the HANDLS database among those participants (n= 1543) who had a baseline Mini-Mental State Examination total score and two baseline 24-hour dietary recalls.

Results. No relationship was observed between absolute n-3 or n-3/n-6 ratios of fatty acid intakes and cognitive decline in this sample, except for the ratio of n-3 PUFA/n-6 PUFA, which showed a significant negative relationship with Mini-Mental State Examination score rate of cognitive change.

Conclusion. Only one of the relationships tested using the 4 dietary predictors for the 11 cognitive outcomes, was significant. Due to only finding one predictor significant for one outcome, it is likely a type-I error.

Chapter 1

INTRODUCTION

Clinical deficits in cognitive function or performance can be observed along a continuum from subtle self-reported changes, to mild cognitive impairment, and to dementia. Cognitive decline may progress over time at nonlinear rates and in various cognitive domains (Lezak, Howieson, Bigler, & Tranel, 2012). By 2050 the prevalence of dementia in the U.S. is projected to nearly triple to 13.8 million (Hebert, Weuve, Scherr, & Evans, 2013). Dementia and cognitive decline are serious health problems, in which age, genetics and environmental factors play a role. Evidence supports an association of dementia and cognitive decline with decreased consumption of omega-3 (n-3) fatty acids in the western diet, but research findings are inconsistent (Cole, Ma, & Frautschy, 2009; Devore et al., 2009; Simopoulos, 2011).

The metabolic pathways of n-3 fatty acids and omega-6 (n-6) fatty acids are mutually competitive, with predominantly antagonistic physiological effects. Because of this physiological opposition, the ratio of n-3 to n-6 fatty acids may be an even better indicator of essential fatty acid status. Limited evidence also supports a positive association between the n-3/6 fatty acids ratio and cognitive decline, dementia and Alzheimer's disease; however, most studies fail to evaluate this ratio (Loef & Walach, 2013).

Furthermore, disparities in cognitive decline and risk for cognitive impairment have been observed between African Americans and Whites (Koyama et al., 2015; Moody-Ayers, Mehta, Lindquist, Sands, & Covinsky, 2005; Zhang, Hayward, & Yu,

2016). Studies suggest that there may be racial differences in diet and genetic polymorphisms related to n-6 fatty acid metabolism (Beydoun et al., 2015; Chilton et al., 2014; Mathias et al., 2011; Raffensperger et al., 2010). These differences could potentially influence disparities in cognitive decline, yet few African Americans have been included in these studies of cognition with n-3 fatty acids and n-3/6 fatty acid ratios (Loef & Walach, 2013). Subtle decline in cognitive function has been reported in middle aged adults, prior to the clinical diagnosis of dementia, at a time when interventions may potentially be more impactful (Petersen, 2009). Evidence for the protection of absolute and relative n-3 fatty acids against cognitive decline and dementia is promising, though inconsistent. Further research is needed before n-3 fatty acid and balanced n-3/6 fatty acid ratios can be widely promoted as an effective, albeit safe, convenient and relatively low-cost intervention to prevent or reduce cognitive decline and dementia among biracial populations. The present study proposes to fill some of these gaps by examining the relationship of dietary intakes of n-3 fatty acids and n-3/6 fatty acid ratios with cognitive decline among a middle-aged biracial urban-dwelling sample.

Chapter 2

LITERATURE REVIEW

2.1 Cognitive Decline and Dementia

Cognitive decline and dementia are rising personal and public health concerns as the population ages. Cognitive decline, with its progressive deterioration of memory and other cognitive domains, can lead to the debilitating condition of dementia, the accompanying incapacity to function independently, and decreased longevity.

The statistics are alarming. Worldwide in 2010 dementia afflicted 35.6 million individuals (Prince et al., 2013). Dementia is a heterogeneous condition, with Alzheimer's disease accounting for about 60-80% of all cases (Gillette-Guyonnet, Secher, & Vellas, 2013; Prince et al., 2013). In 2016 in the United States an estimated 5.2 million people age 65 and older, and 200,000 individuals under age 65 were afflicted with Alzheimer's disease (Alzheimer's Association, 2016; Hebert et al., 2013). That is one in 9 Americans age 65 years and older who has Alzheimer's disease, two-thirds of whom are women (Alzheimer's Association, 2016). For every five years over age 65, the risk of dementia and Alzheimer's disease doubles (Brookmeyer et al., 2011). As the U.S. population ages, the prevalence of late onset dementia and Alzheimer's disease is projected to nearly triple to 13.8 million Americans by 2050, unless measures to prevent, delay or treat dementia are implemented (Hebert et al., 2013).

A recent encouraging Health and Retirement Study among a nationally representative prospective cohort of 21,057 U.S. adults ages 65 and older observed a decrease in dementia prevalence from 11.6% in 2000 to 8.8% in 2012: a 2.8% absolute

decrease and a 24% relative decrease in dementia prevalence. Improved treatments for cardiovascular risk factors and higher levels of education were believed to partially account for the reduction (Langa et al., 2017).

Not only does Alzheimer's disease cause suffering of the afflicted and their families, but it contributes to the soaring cost of healthcare. The estimated national direct cost of dementia was $236 billion in 2016, amounting to about one in five Medicare dollars (Alzheimer's Association, 2016).

Cognitive function ranges across a continuum from normal to a spectrum of deficits from self-reported cognitive decline, to an intermediate precursor state of mild cognitive impairment (MCI), and ultimately dementia (Lezak et al., 2012; Petersen, 2004). Cognitive capability may decline at nonlinear rates and over various cognitive domains (Lezak et al., 2012). Evidence is mounting that self-reported cognitive decline and MCI may be prodromal of Alzheimer's disease (Albert et al., 2011; Donovan et al., 2014; Grundman et al., 2004; Jessen et al., 2014). MCI is characterized by isolated memory loss that is more than expected based on age and education, yet with ability to continue functioning well, without clinical dementia or other cognitive deficits (Petersen, 2009; Petersen et al., 2001). Studies suggest that up to 80% of individuals with MCI may decline to clinically probable Alzheimer's disease over 6 years, at a rate of 8-15% per year. In comparison, normal community-dwellers may decline to Alzheimer's disease at a rate of only 1-2% per year (Grundman et al., 2004; Petersen, 2004). Evidence supports that the onset of neurodegeneration leading to Alzheimer's disease begins decades before overt dementia is manifested (Petersen, 2009; Shaw, Korecka, Clark, Lee, & Trojanowski, 2007). Furthermore, the consensus is that prevention and treatment at earlier stages

has the greatest potential to be effective (Cummings, Doody, & Clark, 2007; Selkoe, 2012).

Although a "cure" for Alzheimer's disease is not yet on the horizon, even methods to delay onset and slow progression would significantly reduce the prevalence and devastation of dementia. Sloane and colleagues (2002) projected that the total number of Alzheimer's disease cases between 2010 to 2050 could be decreased by 35% if the onset of Alzheimer's disease could be delayed by 6.7 years. On the other hand, even if the onset could not be delayed, if progression were slowed, at least the severity of disease in affected individuals would be reduced, potentially requiring less intensive and costly care, and improved quality of life (Cunnane, Chouinard-Watkins, Castellano, & Barberger-Gateau, 2013; Petersen, 2009; Sloane et al., 2002).

Drugs currently have limited effectiveness in the treatment of dementia (Alzheimer's Association, 2016; Solfrizzi et al., 2010). Although age and genetics, such as presence of the ApoEε4 allele, are important risk factors for dementia, other potentially modifiable lifestyle factors, including dietary habits, physical activity, education, and comorbidities, such as diabetes, obesity and cardiovascular disease can also have a major impact on the pathogenesis of dementia (Alzheimer's Association, 2016). In the absence of successful treatment, identification of effective modifiable risk factors is increasingly urgent and imperative. Studying their impact across the lifespan, is important not only among older adults with impaired cognition to determine if benefits could be gained in the short term, but also among younger age groups and healthy individuals to assess if earlier healthful lifestyle factors could be

cumulative and potentially provide neuroprotection later in life (Karr, Alexander, & Winningham, 2011).

2.2 Relationships of Absolute and Relative Polyunsaturated Fatty Acids with Cognition

A variety of nutritional factors such as dietary patterns, specific foods, and isolated nutrients have been associated with benefits and decrements to cognitive function, cognitive decline and dementia (Beydoun et al., 2015; Gómez-Pinilla, 2008; Marie F Kuczmarski, Allegro, & Stave, 2014; Morris, Tangney, Wang, Sacks, Barnes, et al., 2015; Pearson et al., 2016; Witte, Kerti, Margulies, & Flöel, 2014; Wright et al., 2016). Among those that have garnered much support for their neuroprotective benefits over the past few decades are the n-3 fatty acids, particularly the long-chain, highly unsaturated forms, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3), which are found primarily in seafood (Domenichiello, Kitson, & Bazinet, 2015; Dyall, 2015).

2.2.1 Dietary Intakes and Metabolism of Polyunsaturated Fatty Acids

As the prevalence of neurologic and mental disorders has increased, there has been a simultaneous reduction over the past 100 years in the Western dietary consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs), with an absolute and relative increase in n-6 fatty acid intake (Blasbalg, Hibbeln, Ramsden, Majchrzak, & Rawlings, 2011; Simopoulos, 2011). These changes have resulted from decreased consumption of fish and plant products rich in n-3 fatty acids, combined with changes to animal feeds higher in n-6 fatty acids, and industrial production of vegetable oils, most dramatically a greater than 1000-fold increase in soybean oil (Blasbalg et al., 2011; Simopoulos, 2011). A significant percentage of Americans do not meet the

current guidelines for n-3 fatty acid intake (Papanikolaou, Brooks, Reider, & Fulgoni III, 2014). Consequently, there has also been a drastic change in the ratio of n-3 to n-6 FAs, currently as much as 1:25, compared to the pre-industrial balanced dietary ratio of 1:1 (Simopoulos, 2011).

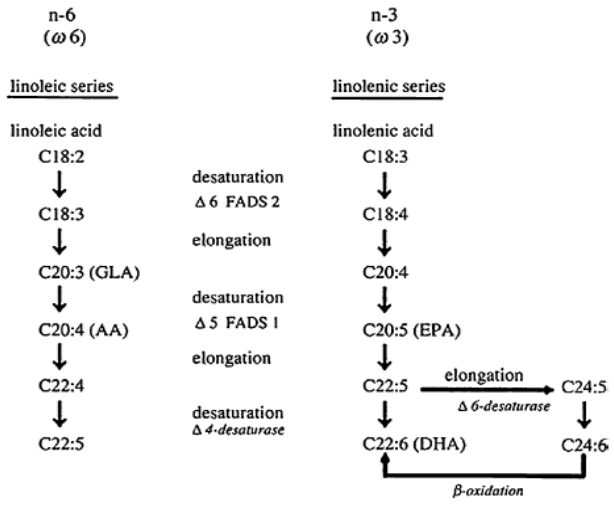
The necessity of the 18-carbon PUFAs, n-3 α-linolenic acid (ALA; 18:3n-3) and n-6 linoleic acid (LA; 18:2n-6) for normal human growth and development was discovered almost a century ago (Burr & Burr, 1930; Simopoulos, 2011). Since these cannot be synthesized de novo by vertebrates, they must be ingested in the diet, and are considered "essential fatty acids". They can be found in plants and their role is primarily as substrates for the biologically active long chain, highly unsaturated fatty acids (HUFAs: > 18 carbons and >3 double bonds).

The HUFA status in the mammalian body is derived by directly ingesting HUFA-rich animal products: in the case of n-3, fish and algae; and n-6, animal meats and products. Alternatively, 20-C and 22-C HUFAs can be metabolized endogenously in the liver tissue from the dietary plant-based 18-C precursors in a process catalyzed by desaturase and elongase enzymes. The ALA which is most concentrated in flaxseed, English walnuts, chia seeds, and canola oil, converts to the major n-3 HUFAs, eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). LA found at high levels in most vegetable oils (corn, safflower, sunflower, and soybean), seeds and nuts, is metabolized to the n-6 HUFA, arachidonic acid (ARA; 20:4n-6). The HUFAs are then secreted as phospholipids and transported throughout the body in lipoproteins. Conversion of essential n-3 and n-6 fatty acids to their HUFA counterparts is limited in humans. Based on isotope-labeled ALA studies, estimates are that only 8-21% of

ALA converts to EPA, and <1-9% to DHA (Childs et al., 2014). Age, sex, habitual dietary food sources, body mass index (BMI) and genetic factors modify conversion rates (Childs et al., 2014). It is controversial therefore, to what extent an adequate DHA and EPA status can be maintained by conversion of plant-derived ALA intake, which depends on individual factors, as well as the intake of LA (Brenna, Salem, Sinclair, & Cunnane, 2009; Welch, Shakya-Shrestha, Lentjes, Wareham, & Khaw, 2010). Recent studies have illustrated that the current U.S. average LA intake of 6 %Energy (E) allows for little conversion of ALA to n-3 HUFA's, so body tissue accumulation would be derived primarily from intakes of EPA and DHA, rather than conversion of dietary ALA (Bibus & Lands, 2015; Lands, Bibus, & Stark, 2017).

Figure 1 shows the metabolic pathways of n-6 LA and n-3 ALA fatty acids are separate and distinct (Simopoulos, 2011). Yet the two groups are catalyzed in the liver by the same enzymes, so their desaturation and elongation are mutually competitive.

Figure 1. Pathways of linoleic and alpha-linolenic acid desaturation and elongation. FADS2 and FADS1 represent genes encoding desaturases 6 and 5 (Simopoulos, 2011).



Because of these competitive pathways, changing dietary intake along one leg of the pathway can change the status of the other (Lands et al., 2017; Simopoulos, 2009; Wood, Mantzioris, Gibson, Ramsden, & Muhlhausler, 2015). For instance, it has been demonstrated that by simply decreasing dietary LA intake from 4.6%E to 2%E ($P < 0.001$) for 4 weeks in healthy adults (18-65 y), without increasing dietary n-3 HUFA intake, significantly increased n-3 HUFA (EPA, DPA, and DHA) and total n-3 PUFA content (6.22% vs. 5.53%, $P<0.001$) was achieved in plasma phospholipids compared to baseline (Wood et al., 2014, 2015). Likewise, rats attained peak plasma levels of phospholipid DHA (>8% total fatty acids) when fed a narrow dietary range of 1–3 %E ALA and 1–2 %E LA, and conversely, when dietary intakes of total polyunsaturated fatty acids (PUFA) exceeded 3 %E, DHA was inhibited to basal levels (∼2% total fatty acids) (Gibson, Neumann, Lien, Boyd, & Tu, 2013). Cleland and colleagues (1992) found that in healthy men, high dietary LA inhibited neutrophil membrane incorporation of EPA compared to a low LA diet . In healthy men who had constant 1% E ALA intake, LA intake was inversely associated with plasma phospholipid EPA (Liou, King, Zibrik, & Innis, 2007). On the other hand, DHA supplementation decreased ARA concentrations in RBCs and plasma of healthy men (Schuchardt et al., 2016). Therefore, the ratio of n-3 to n-6 fatty acids may be an even better indicator of essential fatty acid status than absolute levels.

2.2.2 **Functions of Polyunsaturated Fatty Acids in the Brain**

The n-3 HUFAs DHA and EPA, as well as the n-6 fatty acid arachidonic acid (ARA; 20:4n-6), are essential for membrane structure and fluidity, vision, neurotransmission, neuroinflammation, cell survival, in the regulation of gene expression, and as precursors to eicosanoids and other cell signaling molecules

(Guesnet & Alessandri, 2011; Simopoulos, 2011). The PUFAs accumulate in tissues, primarily in the second position of phospholipids.

Neuronal and glial brain cell membranes are highly enriched with DHA, comprising up to 25% (weight %) of the brain phospholipids, at least 30% of the retina and 60% of the rod photoreceptors (Guesnet & Alessandri, 2011). Therefore, adequate DHA is required for normal neurological development, and is vital throughout the lifespan (Guesnet & Alessandri, 2011; Stonehouse, 2014). The n-3 and n-6 fatty acids also act as natural ligands for nuclear receptors, and can directly or indirectly alter transcription of genes related to energy metabolism, inflammation, platelet derived growth factor and others (Simopoulos, 2009; Szostak et al., 2016).

Eicosanoid signaling molecules derived from the 20-C n-3 EPA and n-6 ARA are divided into four main groups: the thromboxanes, leukotrienes, prostaglandins, and prostacyclin. Most tissues have an array of selective eicosanoid receptors for regulation of functions essential for health (Lands, 2015). The cell-signaling end-products of ARA (the n-6 eicosanoid precursor) however, are predominantly antagonistic to products of EPA (the n-3 eicosanoid precursor) in their physiological effects (Simopoulos, 2011). The ARA-derived molecules are primarily vasoconstrictive, pro-inflammatory, thrombotic, pro-arrhythmic and angiogenetic, while the EPA-derived molecules have a less intense or direct opposing effect (Janssen & Kiliaan, 2014; Lands, 2015; Simopoulos, 2011). Therefore, a shift of balance toward the ARA cascade can influence physiology in a pathological way (Lands et al., 2017). For example, ARA derived eicosanoids produce the cytokines, tumor necrosis factor-α, IL-1, and IL-6, which are pro-inflammatory and involved in immunity (Simopoulos, 2011). ARA derived prostaglandin cell signaling has been

shown to affect brain structure and function in potentially harmful ways. For instance, in a study of Alzheimer disease model mice, ARA derived prostaglandin E2 receptor EP2 signaling suppressed beneficial microglial maintenance functions (Johansson et al., 2015). These regenerative processes control inflammation, prevent accumulation of β-Amyloid42 peptides, and promote insulin growth factor-1 regulated synaptogenesis, neurogenesis, and neuroprotection (Johansson et al., 2015). Further, ablation of the EP2 signaling prevented cognitive impairment and synaptic protein loss in these mice. An imbalance of n-6 HUFA leads to over-reaction of the ARA cascade, which can influence brain structure and function in harmful ways, and can be lessened by n-3 HUFA mediators (Lands, 2015; Lands et al., 2017).

2.2.3 Human Observational Studies for Omega-3 PUFAs and Cognition

The health benefits of dietary n-3 fatty acids, EPA and DHA were first noted among the Greenland Eskimos, who ate a diet of whale and seal meat, rich in marine n-3 fatty acids, and had a low incidence of cardiovascular diseases (De Caterina & De, 2011; Simopoulos, 2010). Since then, benefits of n-3 fatty acids have been expanded to include cancer, depression, and other chronic inflammatory and autoimmune diseases (Simopoulos, 2011). A growing body of research has shown an association of n-3 PUFA intake with cognitive decline and dementia, but research findings have been inconsistent (Cole et al., 2009; Devore et al., 2009; Luchtman & Song, 2013).

Most animal studies demonstrate a strong effect of n-3 PUFA depletion and supplementation on cognitive function in healthy and Alzheimer's disease models (Agrawal & Gomez-Pinilla, 2012; Hooijmans, Pasker-de Jong, de Vries, & Ritskes-Hoitinga, 2012; Luchtman & Song, 2013). Numerous human cross-sectional studies show that n-3 PUFA or fish consumption is beneficial to cognition. Fish intake was

negatively associated with dementia prevalence across the globe from Latin Americas to India and China among 14,960 adults ≥age 65 years (Albanese et al., 2009). Increased dietary HUFAs were associated with decreased risk of cognitive decline in verbal fluency over 6-years (OR 0.79, 95% CI 0.66–0.95) among adults ages 50-65 years in the Atherosclerosis Risk in Communities (ARIC) study (Beydoun, Kaufman, Satia, Rosamond, & Folsom, 2007; Beydoun, Kaufman, Sloane, Heiss, & Ibrahim, 2008). Higher fish and PUFA consumption or higher serum HUFAs were associated with better cognitive performance, semantic memory and verbal fluency (D'Ascoli et al., 2016; Eskelinen et al., 2008; Phillips, Childs, Calder, & Rogers, 2012), while others showed no association (Kroger et al., 2009).

Most, though not all, longitudinal studies show that higher intakes of fish, and higher intakes or blood markers of n-3 PUFAs or n-3 HUFAs have been associated with neuroprotection, slower rate of cognitive decline over multiple domains, decreased incident Alzheimer's disease, or decreased all-cause dementia risk (Beydoun et al., 2007, 2008; Fotuhi, Mohassel, & Yaffe, 2009; Morris et al., 2003; Morris, Evans, Tangney, Bienias, & Wilson, 2005; Solfrizzi et al., 2010; van de Rest et al., 2016; van Gelder, Tijhuis, Kalmijn, & Kromhout, 2007). A few inconsistent studies have shown no association (Devore et al., 2009; Kroger et al., 2009), while one study deviated from the others in their observation of worse cognitive outcomes with higher n-3 plasma PUFA status (Laurin, Verreault, Lindsay, Dewailly, & Holub, 2003). The Canadian Study of Health and Aging (CSHA) found higher mean baseline plasma phospholipid concentration of EPA ($p < 0.01$) in cognitively impaired cases, and higher DHA ($p < 0.07$), n-3 PUFA ($p < 0.04$) and total PUFA ($p < 0.03$) concentrations in dementia cases compared to controls (Laurin et al., 2003).

Nevertheless, the later CSHA study with a larger sample observed no association with red blood cell PUFAs and dementia or AD (Kroger et al., 2009; Laurin et al., 2003).

2.2.4 Human Interventional Trials for Omega-3 HUFAs and Cognition

Some n-3 HUFA supplementation trials have shown promise for neuroprotection, however evidence for benefit is inconclusive (Cole et al., 2009; Stonehouse, 2014). Inconsistencies in treatment duration, dosage, varying formulations, assessment of baseline status of n-3 and n-6 PUFAs, evaluation of sustained effect after treatment, and comparison to control groups can all limit interventional studies (Cooper, Tye, Kuntsi, Vassos, & Asherson, 2015; Maclean et al., 2005).

Numerous trials among healthy older adults have shown improved cognitive performance with n-3 HUFA supplementation (Stonehouse, 2014). In a cross-over placebo study of healthy adults (n=40) ages >50 y, 3 grams/day n-3 HUFA fish oil supplementation for 5 weeks improved performance on working memory tests and lowered systolic BP compared with placebo (Nilsson, Radeborg, Salo, & Björck, 2012). A randomized double-blind placebo-controlled trial involving 65 healthy adults ages 50-75 years treated with 2.2 grams/day n-3 HUFA fish oil over 26 months showed improved executive function and also enhanced structural and functional brain changes on MRI compared to placebo (Witte, Kerti, Hermannstädter, et al., 2014). A double-blind, placebo-controlled trial by van de Rest and colleagues (2008) studied 302 cognitively healthy adults (Mini-Mental State Examination score > 21) ages 65 years or older across multiple cognitive domains. Individuals were randomized to 26-weeks of high-dose (1,800 mg/d) EPA–DHA, low-dose (400 mg/d) EPA–DHA, or placebo capsules. Compared with placebo there was an improvement in the cognitive

domain of attention for both low and high EPA–DHA supplementation groups observed in carriers of the APOEε4 allele, and for the low EPA–DHA supplementation in men (van de Rest et al., 2008).

Studies among adults with age-related cognitive decline (Yurko-Mauro et al., 2010) or MCI, (Chiu et al., 2008; Lee, Shahar, Chin, & Yusoff, 2013) showed supplementation with n-3 HUFA significantly improved memory compared to controls, but among those with Alzheimer's disease there was no improvement after 24 weeks (Chiu et al., 2008).

2.2.5 Human Studies for Omega-3/-6 Fatty Acid Ratios and Cognition

Since n-3 fatty acids and n-6 fatty acids metabolism is competitive, while the physiological effects are primarily opposed, it is more valuable to evaluate cognition in relation to the relative levels of n-3 and n-6 fatty acids, rather than absolute levels. Indeed, limited observational evidence also supports an association between higher n-3/6 fatty acid intake ratios with less cognitive decline, dementia and AD; however, most studies fail to evaluate this ratio (Loef & Walach, 2013).

Two cross-sectional studies assessed n-3/n-6 fatty acid plasma ratios in participants with cognitive decrements. The InCHIANTI Study group in Italy found lower n-3/n-6 fatty acid plasma ratios, lower n-3 total fatty acid and ALA levels in adults with dementia, compared to cognitively healthy adults ages 65 and older (Cherubini et al., 2007). Another study by Conquer and colleagues (2000)found participants with cognitive impairment/no dementia (CIND), Alzheimer's dementia and other dementia had lower plasma levels of the n-3/n-6 ratio, EPA, DHA, and total n-3 fatty acids in total phospholipid and phosphatidylcholine fractions, and lower n-3 fatty acids in other fractions, compared to healthy controls (Conquer et al., 2000).

A cross-sectional study of 286 cognitively intact Japanese adults evaluated relationships of serum EPA/ARA and DHA/ARA ratios with brain MRI white matter hyperintensity and cognitive function on the Mini-Mental State Examination (Suwa, Yamaguchi, Komori, Kajimoto, & Kino, 2015). The progression of white matter hyperintensity grade from none or mild to advanced, was related to low serum EPA/ARA ratio (<0.38) and low serum EPA, but not to low serum DHA/ARA ratio. White matter hyperintensity grade was also related to reduction of function on the Mini-Mental State Examination, suggesting that sufficient n-3HUFA intake may potentially protect against cognitive deficits.

Prospective studies evaluating the association of the dietary n-3/n-6 ratio with cognitive decline or dementia are scant. The Three City Cohort study in France, found increased fish intake protective against Alzheimer's disease and all cause dementia, the latter only among APOEε4 non-carriers (Barberger-Gateau et al., 2007). Dementia risk was 60% less in individuals with habitual intake of n-3 rich oil, with no genotypic interactions. While not explicit in their analysis of the ratio, habitual intake of n-6 vegetable oil, uncompensated by n-3 oils or fish intake, was associated with increased dementia risk over 3.5 years, but only among non-carriers of the APOEε4 allele (HR 2.12, 95% CI: 1.30, 3.46, p = 0.003) (Barberger-Gateau et al., 2007). Another group found a higher n-6/n-3 intake ratio associated with higher risk of recent cognitive decline (highest to lowest group OR = 1.25; 95% CI 1.01-1.55) (Vercambre, Boutron-Ruault, Ritchie, Clavel-Chapelon, & Berr, 2009).

Likewise, a paucity of longitudinal studies has evaluated blood marker ratios of n-3/n-6 fatty acids in relation to cognitive outcomes. The ARIC study found higher ratios of n-3/n-6 HUFAs and absolute n-3 HUFA levels, both in plasma cholesteryl

esters and phospholipids and in the diets, were associated with significantly lower risk of cognitive decline for the word fluency test over 6 years among all subjects and hypertensives (Beydoun et al., 2008). Another group evaluated erythrocyte membrane ratios of n-3 HUFAs/n-6 PUFAs, and DHA/ARA in relation to 4-year cognitive decline on the Mini-Mental State Examination among 246 adults ages 63-74 years. Lower ratios of n-3/n-6 PUFAs, and DHA/ARA, and absolute levels of DHA and EPA were found in the "decline" group, compared to the "no decline" group (Heude, Ducimetiere, & Berr, 2003).

Pase and colleagues (2015) randomized 160 healthy adults ages 50-70 years, in a double-blind placebo-controlled supplementation trial, to 16-weeks of treatment with 3 g/d fish oil (240 mg EPA + 240 mg DHA) plus multivitamin, 6 g/d fish oil with or without multivitamin or placebo. Compared to placebo, all treatment groups had increased erythrocyte EPA/ARA ratios, no groups had increased DHA, only the two high dose groups had increased EPA, and only the group receiving 6 g of fish oil without multivitamins had decreased erythrocyte n-6 levels compared to placebo. Increases in the erythrocyte n-3/6 ratio were associated with improvements in spatial working memory response times irrespective of the assigned treatment (Pase et al., 2015).

2.3 Summary

Evidence for the protection of absolute and relative n-3 fatty acids against cognitive decrements is promising, but more research is needed to determine if increased dietary intake of highly-unsaturated n-3 fatty acids and balanced n-3/6 fatty acid ratios can be widely promoted as an effective, albeit safe, convenient and relatively low-cost intervention to prevent or reduce cognitive decline and dementia

among biracial populations. This study proposes to address some of the gaps in the literature regarding the relationship of n-3 fatty acids and their ratios in a community setting involving a biracial and socioeconomically diverse sample of middle-aged adults.

2.4 Research Aims and Hypotheses

The primary aim of this study is to evaluate the relationship of absolute n-3 fatty acid dietary intake and relative n-3/n-6 fatty acid dietary intake with cognitive decline in an urban population of socio-economically diverse African American and white adults.

It was hypothesized that adults with higher absolute n-3 fatty acid dietary intakes or higher ratios of omega-3/6 fatty acid intakes would have lower levels of cognitive decline over the period of data collection.

Chapter 3

METHODS

3.1 HANDLS Database Background

The data source was the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS), a 20 year longitudinal, multidisciplinary epidemiological study of health-related disparities among biracial, community-dwelling urban adults (Evans et al., 2010) (Evans, M. K., Lepkowski, J. M., Powe, N. et al 2010). HANDLS participants comprise a fixed cohort of 3720 socioeconomically diverse African American and white men and women ages 30-64 years old. Participants were recruited at home-based screenings from thirteen contiguous neighborhoods in Baltimore city based on census tracts (year 2000 data). The selected areas were expected to yield distributions representative of Baltimore City in a four-way factorial cross, for race, sex, age (seven 5-year strata) and socioeconomic status (household income self-reported as above or below 125% of the Health and Human Services Poverty Guidelines:2004). Five waves of data collection have been planned for the study duration (2004-2024), with Wave 4 data being collected currently. Subjects are compensated monetarily.

3.2 HANDLS Wave 1 and Wave 3 Protocols

Baseline data for Wave 1 (accrued 2004-2009) were collected in two phases. In the first phase, residences within pre-determined neighborhoods were identified. Interviewers screened for eligibility at the doorstep, and invited eligible candidates to participate in HANDLS. Additional eligibility requirements were ability to give informed consent, and possession of a valid photo I.D. Individuals were excluded for cancer treatment within 6 months prior to recruitment, and pregnancy. At initial

contact, consenting participants completed an in-home survey and the first 24-hour dietary recall.

Phase 2 baseline data collection was done by trained personnel on Medical Research Vehicles (MRVs), parked in the participant's neighborhood, and consisted of a second 24-hour dietary recall, and multidisciplinary biomedical, demographic, psychosocial, neurocognitive and genetic evaluations. After enrollment, participants were excluded who were medically unfit for participation in the study (for example, uncontrolled blood pressure > 160/100 mmHg or acute drug or alcohol intoxication). Seventy-five percent (n=2802) of consented participants completed the baseline assessments.

Wave 2 was an interim assessment primarily for development and validation of retention and re-contact strategies, and collection of supplemental personality domain data (Revised NEO Personality Inventory) (Zonderman, Evans, & Costa, 2006). Wave 3 (2009-2013) was the first multidisciplinary follow-up evaluation of the entire HANDLS cohort. Data collection took place in two different settings. For participants who could come to the MRV, data were collected in sessions. The first session occurred on the MRV where the first dietary recall was done in person, along with a medical examination, cognitive testing, and several other measures a such as the fasting blood draw and body composition measures. The second session consisted of the second dietary recall and dietary supplement questionnaire which were done by phone. Home study visits were arranged for participants who moved out of Baltimore or who live in Baltimore but were immobile. For more details see Evans, et al., (2010) or the HANDLS website http://handls.nih.gov/.

3.3 Study Design

Our present study is a prospective longitudinal design to examine the relationship of baseline absolute n-3 fatty acid and relative n-3/n-6 dietary intakes with cognitive test performance change from Wave 1 to Wave 3 in a secondary analysis of the HANDLS database among the participants who had two baseline 24-hour dietary recalls, and a baseline Mini-Mental State Examination total score (MMStot) (Folstein, Folstein, & McHugh, 1975). Exclusions were for those with medical history of stroke, TIA, and HIV, and low serum levels of vitamin B12 (<200 pg/mL) and folate (<3.0 ng/mL), which are established causes of cognitive impairment.

The participants gave informed consent, and the Institutional Review Board of the National Institute of Aging at the MedStar Research Institute approved the HANDLS study. This study was also approved by the Institutional Review Board at the University of Delaware (Appendices A.1, A.2, A.3).

3.4 Dietary Assessment, Omega-3 and Omega-6 Fatty Acid Exposures

Dietary assessments were based on the means of two baseline 24-hr dietary recalls collected by trained interviewers using the USDA Automated Multiple Pass Method (AMPA) (Blanton, Moshfegh, Baer, & Kretsch, 2006). The 5-step AMPA is a standardized and validated quantitative process for recall of carbohydrate, fat, protein, and energy consumption, using food models, measuring cups and spoons to estimate portions (Conway, Ingwersen, & Moshfegh, 2004; Conway, Ingwersen, Vinyard, & Moshfegh, 2003; McBride, 2001). Food intakes were coded by trained nutrition professionals using the USDA Food and Nutrient Database for Dietary Studies, version 3, for estimation of nutrient consumption (Ahuja, Moshfegh, Holden, & Harris, 2013).

Mean baseline dietary intakes of each of the individual n-3 fatty acids and n-6 fatty acids for two 24-hour recalls were adjusted for energy and expressed as a percentage of total energy intake. Then the sums were calculated for total n-3 polyunsaturated fatty acids (n-3PUFAs: ALA + EPA + DPA + DHA), n-3 highly unsaturated fatty acids (n-3HUFAs: EPA + DPA + DHA), and n-6 polyunsaturated fatty acids (n-6PUFA: LA + ARA). Dietary exposures were then expressed as total n-3 polyunsaturated fatty acids (n-3PUFAs) or n-3 highly unsaturated fatty acids (n-3HUFAs), and as ratios of n-3HUFA/ n-6PUFA, and n-3/n-6PUFA.

3.5 Cognitive Outcomes

Cognitive function was assessed at Waves 1 and 3 by a battery of standardized and validated neurocognitive tests measuring performance over multiple cognitive domains.

3.5.1 Global Cognitive Ability

The Mini-Mental State Examination screens for general cognitive function by assessing attention, memory, language and orientation to time and place (Folstein et al., 1975). Mini-Mental State Examination total scores (MMStot) from 0-30 denote the number correct, so higher scores represent better performance. Although low scores on the MMStot are usually indicative of brain dysfunction, low scores are also significantly associated with age and poor education or related factors, such as poor test taking skills. For this reason, MMStot may be unable to distinguish dementia-like functioning from poor overall cognitive development that may be associated with poor literacy or low educational achievement (Crum, Anthony, Bassett, & Folstein, 1993; Uhlmann & Larson, 1991)

3.5.2 Verbal Learning and Memory

The California Verbal Learning Test-II (CVLT-II) utilizes word-list learning trials to measure verbal memory and learning (Delis, Kramer, Kaplan, & Thompkins, 2000). In the CVLT, two 16-word lists are used to assess total words correct during short-term and delayed free recall. Higher scores signify greater verbal learning and memory function.

3.5.3 Visual Memory

The Benton Visual Retention Test-5th edition (BVRT) measures short-term visual memory and perception (Sivan, 1992). In the BVRT Administration A, Form D, used for HANDLS, each of ten designs was revealed for 10 seconds, followed by the participant attempting to accurately draw the design on paper from memory. Independent scoring of errors by two trained evaluators was based on the BVRT Manual, with consensus reached in cases of discrepancy. Since the scores are determined by the number of errors, higher scores indicate worse visual memory performance.

3.5.4 Working Memory and Attention.

The Digit Span Forward and Digit span Backward are components of the Wechsler Adult Intelligence Scale, Revised (WAIS-R) that measures attention, short-term auditory memory, executive processes and working memory (Weschler, 1981). The participant is asked to repeat a series of 3-9 numbers forward and 2-9 numbers in reverse order. The score is the total number of correct trials forward and backward, so higher numbers represent better performance.

The Brief Test of Attention (BTA) measures auditory divided attention, described as two selective auditory attention tasks performed simultaneously

(Schretlen, 1989). Ten lists of combined numbers and letters (for example, "M-6-3-R-2") of increasing length (from 4-18 characters) were recited to the participant, who was asked to track, without using their fingers, how many numbers were spoken, ignoring the letters. The total score was the number of correct trials with a maximum total score of ten trials.

3.5.5 Cognitive Flexibility and Attention

Trail Making Test A (Trails A) and Trail Making Test B (Trails B) tests measure attention, cognitive control, visuomotor scanning and speed of processing (Reitan, 1992). Participants were asked to draw lines between each of 25 numbers in consecutive ascending order in Trails A, or alternating between consecutive letters and numbers in Trails B as quickly as possible. Scores were time required to complete each test, so higher scores reflected worse performance.

3.5.6 Semantic Fluency

The Animal Fluency Test measures verbal fluency. The participants are instructed to list as many animal words as they can within a 60 second time period (Delis, Kaplan, & Kramer, 2001). Scoring is based on the total number of unique animal words generated, so higher scores reflect greater semantic fluency.

3.5.7 Visuospatial Performance

The Clock Drawing Test measures visuospatial and visuo-constructional performance. For the "clock command" version, subjects were instructed to draw a clock with all numbers and to place the hands to demonstrate 10 minutes past 11 o'clock. Scores were assigned for the clock face (0-2), numbers (0-4) and hands (0-4),

with a possible total of 10 points, with higher scores reflecting better performance (Rouleau, Salmon, Butters, Kennedy, & McGuire, 1992).

3.6 Subject Characteristics

The participant characteristics were measured in the manner discussed below.

3.6.1 Demographic Features

Poverty status, sex, and race were defined from baseline questionnaires. Ages at the time of examination at Wave 1 and Wave 3 were calculated from reported date of birth. Years of education completed was collected as part of the household baseline questionnaire. Current use of hard drugs, names marijuana, heroin, cocaine, was collected in the baseline medical history.

3.6.2 Literacy Measures

The Wide Range Achievement Test- Revision 3 (WRAT) Letter and Word Reading subtest for ages 5 to 75 years was the baseline literacy assessment used in the HANDLS study (Wilkinson, 1993). Trained evaluators gave participants a list of 42 increasingly difficult words to read. A basal score of 15 was added for letter reading, if the subject could correctly read the first five words.

3.6.3 Clinical Measures

BMI was calculated as weight in kilograms per height in meters^2. Calibrated equipment used for obtaining anthropometric measurements were the Health O Meter Digital Lithium Scale (Model #HDL 976) for weight, and the Novel Products Inc. Height Meter (Model #DES 290237) for height. The brachial artery blood pressure was measured in each arm by standard auscultation method after resting 5 minutes

while seated. The two systolic blood pressures were averaged for the seated systolic blood pressure value in mm Hg.

3.6.4 Depressive Symptoms

Depression was measured by the Center for Epidemiologic Studies-Depression score (CES-D), a short self-report questionnaire designed and validated for use among the general population in non-clinical settings (Radloff, 1977).

3.7 Statistical Analysis

Data analyses were conducted using Stata software, version 14.0. Significance was defined as p values <0.05.

Only participants with baseline MMStot scores, who had two baseline 24-hour dietary recalls were included in the analysis. Individuals were excluded from the present study for medical history of stroke, TIA and HIV, low vitamin B12 (<200 pg/mL) and folate (<3.0 ng/mL) serum levels, which are established causes of cognitive impairment in the literature.

Adjustments were made in the analysis for baseline demographic and lifestyle variables of age, sex, race, poverty status, educational level, literacy measured by the WRAT, cigarette smoking, hard drug and alcohol status. CES-D scores, as well as potential cardiovascular and metabolic confounders of BMI, seated systolic BP, and diabetes status at baseline were also included in the models.

3.7.1 Demographic Variables

Age was a continuous measure of years at baseline. Sex was coded 0= female; 1= male. Race was self-reported as 0= White, or 1= African American. Poverty status

was coded dichotomously as either above (=0) or below (=1) 125% of the HHS Federal Poverty Guidelines for 2004.

3.7.2 Educational Achievement and Literacy

Educational achievement was designated by the years of formal education completed at baseline (e.g., high school = 12 years; attainment of college bachelor's degree = 16 years).

3.7.3 Lifestyle and Cardiovascular Risk Variables

Dichotomous codes were assigned for lifestyle substance use variables of cigarette smoking, alcohol, and hard drug status at baseline. Smoking status was given 0= not current user (never tried, never used regularly, or former users) and 1= current user. Alcohol status was assigned 0= not current user (never tried, never used regularly, or used >6 months ago) and 1= used within the past 6 months. Lastly, hard drug use, regarded as marijuana, opiate, or cocaine use, was assigned 0= not current user (never tried, never used regularly, or used >6 months ago) or 1= used in the past 6 months.

Diabetes status at baseline was regarded positive for fasting blood glucose ≥ 126 mg/dL, diabetes medication prescription, or a history of diabetes by self-report (1=no, 2=yes).

3.7.4 Cognitive Test Scores and Exposures

Mean cognitive test raw scores between Wave 1 and Wave 3 were calculated for the total sample, and for White and African American groups using linear regression. Differences in mean cognitive test scores between waves, and within each wave between the race groups were tested (significant if Prob > F less than 0.05).

Each neurocognitive test was then analyzed separately for each of the four exposures (n-3PUFA, n-3HUFA, n-3HUFA/n-6PUFA, and n-3/n-6PUFA) in a series of mixed-effects regression models, to assess how each exposure affected the longitudinal rate of cognitive change over time from Wave 1 to 3. Because the rate of change was examined, this method did not distinguish between significant improvement, stability/ no significant change, and decline, since all those categories were treated similarly. To increase statistical power, the mixed-effects regression model used assumed missingness at random, and that it was ignorable. In the model time is categorized as visit 2 (Wave 3) vs visit 1 (Wave 1), where time = 0 at visit 1 and the time elapsed is between the two visits at visit 2.

Models were adjusted for baseline age (center 50), sex, race, poverty status, educational level in years (center 12), WRAT total score (center 40), cigarette smoking, hard drug and alcohol status, BMI (center 30), seated systolic BP (center 120), CES-D (center 15), diabetes status. The Inverse Mills ratio is also included in the regression analysis to adjust for possible selection bias.

Chapter 4

RESULTS

4.1 Sample Characteristics

Baseline descriptive characteristics for the included HANDLS sample (n=1543) are presented in Table 1. The mean participant age was 46.64 years. The cohort included 36.09% white adults (weighted), 53.93% women, and 79.86% had a household income above 125% of the poverty guideline. At baseline, 63.51% of the total sample were current alcohol users, 46.37% were current cigarette users and 18.87% were current hard drug users. Diabetes status was prevalent in 12.66% of the sample. Mean BMI of 29.21 kg/m^2 was in the overweight range, and mean seated systolic BP of 118.89 mm Hg was within normal limits.

Significant baseline differences were observed between race groups. More African Americans than Whites were below 125% of poverty guideline, used cigarettes and hard drugs. African Americans had lower education, higher seated systolic BP, and higher intakes of n-3PUFA (%kcals), n-3HUFA (%kcals), and n-3HUFA/n-6PUFA compared to Whites.

Table 1: Baseline Characteristics of Sample

		Total sample n=1543		Whites n=674		African Americans n=869		
	Observed %	Weighted proportion or mean (SE)	95% CI	Weighted proportion or mean (SE)	95% CI	Weighted proportion or mean (SE)	95% CI	P value between races
Race (white) %	43.68	36.09 (0.0189)	32.47-39.88					
Sex (% females)		53.93 (0.0214)	49.71-58.09	53.88 (0.0283)	48.30-59.36	53.96 (0.0294)	48.16-59.66	0.984
Poverty status (% >125%)		79.86 (0.0131)	77.18-82.31	87.57 (0.0113)	85.17-89.63	75.51 (0.0199)	71.41-79.19	< 0.001
Alcohol Status (% current users)		63.51 (0.0205)	59.42-67.43	68.90 (0.0261)	63.56-73.79	60.47 (0.0285)	54.77-65.90	0.294
Cigarette status (% current users)		46.37 (0.0217)	42.15-50.64	37.57 (0.0265)	32.53-42.89	51.34 (0.0295)	45.56-57.08	< 0.001
Hard Drug status (% current users)		18.87 (0.0179)	15.60-22.63	12.01 (0.0173)	9.01-15.83	22.74 (0.0259)	18.06-28.21	< 0.001
Diabetes status (% yes)		12.66 (0.0136)	10.22-15.57	10.15 (0.0166)	7.32-13.90	14.07 (0.019)	10.72-18.26	0.121
Age Wave 1 (yrs)		46.64 (0.3637)	45.93-47.35	46.20 (0.5200)	45.18-47.22	46.89 (0.4874)	45.93-47.85	0.332
Household education (yrs)		13.02 (0.1311)	12.77-13.28	13.89 (0.236)	13.43-14.36	12.54 (14.82)	12.25-12.83	< 0.001
BMI (kg/m2)		29.21 (0.3272)	28.57-29.85	28.94 (0.3724)	28.21-29.67	29.37 (0.4673)	28.45-30.28	0.475
Wave 1 CES-D		13.60 (0.4220)	12.77-14.43	13.37 (0.5846)	12.19-14.48	13.75 (0.5711)	12.63-14.87	0.616
Mean systolic BP (mm Hg)		118.89 (0.6852)	117.54-120.23	116.42 (0.7919)	114.86-117.97	120.28 (0.9678)	118.38-122.18	0.002
n-3PUFA (%kcals)		0.7384 (0.0173)	0.7045-0.7722	0.6966 (0.0172)	0.6628-0.7303	0.7620 (0.0253)	0.7124-0.8115	0.0325
n--3HUFA (%kcals)		0.1075 (0.0088)	0.0903-0.1247	0.0697 (0.0082)	0.0537-0.0857	0.1288 (0.0129)	0.1034-0.1542	< 0.001
n-3HUFA/ n-6PUFA		0.0174 (0.0019)	0.0137-0.0212	0.0124 (0.0017)	0.0091-0.0157	0.0203 (0.0029)	0.0147-0.0259	0.017
n-3/n-6PUFA		0.1118 (0.0022)	0.1075-0.1161	0.1137 (0.0022)	0.1094-0.1179	0.1107 (0.0032)	0.1045-0.1170	0.4434

4.2 Description of Excluded Sample

For this study 648 individuals were excluded who had fewer than two 24-hour dietary recalls. Exclusions were made for medical history of AIDS/HIV in 76, stroke in 58 and TIA in 77 participants. There were 8 individuals excluded for low serum folate <3.0 ng/ml, and 17 excluded for serum vitamin B12 <200 pg/ml (none of which overlapped with folate <3.0 ng/ml). (Refer to Consort Chart, Appendix B).

No significant differences were observed between the final sample and those excluded from the final sample for baseline characteristics of race, sex, poverty status, or mean age (see Appendix C.1).

4.3 Cognitive Decline Scores

Descriptive statistics were reported in Table 2 and Appendix C.2 for mean raw cognitive test scores in Wave 1 and Wave 3 among the total sample, and stratified by race. Of all individuals with a valid MMStot score who were included, only five had an MMStot score <20.

There were significant decreases in verbal memory and learning performance on the CVLT List A immediate recall ($p< 0.001$) and CVLT List A long-delay free recall ($p< 0.001$) and visual memory performance on the BVRT ($p< 0.001$) between Waves 1 and 3 for total sample, Whites and African Americans.

Significant differences in performance between Whites and African Americans were noted within each of the Waves (Wave 1 and Wave 3) for all mean cognitive test raw scores except for BVRT in Wave 1.

Table 2: Mean (±SE) Cognitive Test Scores for HANDLS Total Sample and by Race

Mean Cognitive Test Raw score ±SE	Total sample		Whites		African Americans		P value between races within each wave	
	Wave 1	Wave 3	Wave 1	Wave 3	Wave 1	Wave 3	Wave 1	Wave 3
MMStot	27.97 ± 0.09	28.04 ± 0.08	28.53 ± 0.08	28.54 ± 0.08	27.65 ± 0.13	27.78 ± 0.11	<0.001	<0.001
CVLtca	25.29 ± 0.34	20.53 ± 0.36 **	27.43 ± 0.50	22.54 ± 0.58 **	24.12 ± 0.44	19.38 ± 0.45 **	<0.001	<0.001
CVLfrl	7.59 ± 0.16	6.04 ± 0.16 **	8.55 ± 0.21	7.18 ± 0.28 **	7.06 ± 0.21	5.41 ± 0.19 **	<0.001	<0.001
BVRTot	5.22 ± 0.21	7.34 ± 0.23 **	4.96 ± 0.25	6.17 ± 0.25 **	5.38 ± 0.30	7.99 ± 0.32 **	0.285	<0.001
Attention	6.75 ± 0.10	6.77 ± 0.10	7.50 ± 0.12	7.28 ± 0.14	6.34 ± 0.14	6.48 ± 0.12	<0.001	<0.001
Fluency Word	19.31 ± 0.25	19.78 ± 0.27	21.54 ± 0.42	21.48 ± 0.44	18.05 ± 0.27	18.87 ± 0.33	<0.001	<0.001
Digit span Fwd	7.43 ± 0.09	7.58 ± 0.11	8.01 ± 0.14	8.26 ± 0.18	7.12 ± 0.12	7.22 ± 0.14	<0.001	<0.001
Digit span Bck	5.83 ± 0.09	5.96 ± 0.10	6.76 ± 0.13	6.76 ± 0.17	5.30 ± 0.11	5.54 ± 0.11	<0.001	<0.001
Clock command	8.81 ± 0.06	8.87 ± 0.06	9.07 ± 0.06	9.00 ± 0.07	8.66 ± 0.08	8.80 ± 0.08	<0.001	0.045
TrailsAtest Sec	34.26 ± 0.72	34.92 ± 1.42	28.82 ± 0.59	29.99 ± 0.90	37.44 ± 1.06	37.63 ± 2.15	<0.001	=0.001
TrailsBtest Sec	126.03 ± 4.75	116.87 ± 6.68	90.17 ± 4.73	79.03 ± 3.37	146.95 ± 6.89	137.99 ± 9.90	<0.001	<0.001

P value between Wave 01 and Wave 03 for each group *p< 0.05, **p< 0.001

Abbreviations: MMStot, Mini-Mental State Exam total score; CVLtca, California Verbal Learning test total correct List A immediate recall; CVLfrl, California Verbal Learning test # correct List A long-delay free recall; BVRTot, Benton Visual Retention test total errors; Attention, Brief test of Attention total correct; Fluency Word, Animal Fluency total words; Digit span Fwd, Wechsler Adult Intelligence Scale-Revised Digit Span Forward total score; Digit span Bck, Wechsler Adult Intelligence Scale- Revised Digit Span Backward total score; Clock command total score; TrailsAtest Sec, Trails A test seconds; TrailsBtest Sec, Trails B test seconds. **Note: For BVRT and the Trails A and B lower numbers are better**

4.4 Relationship of Race, Omega Fatty Acid Variables and Cognitive Decline

In Tables 3A, 3B, 3C, 3D, the relationships between outcomes (cognitive change) and the n-3PUFAs, n-3HUFAs and ratios of n-3HUFA/n-6PUFA, and n-3/n-6PUFA were not significant, with one exception. The ratio of n-3PUFA/n-6PUFA showed a significant negative relationship with Mini-Mental State Examination total score cognitive change from Wave 1 to Wave 3 (b= -0.255, SE 0.125; P>Z 0.042, 95% CI -0.500, -0.009).

Table 3A: Cognitive Change Wave 1 to Wave 3 for n-3PUFA %kcals

Cognitive test	Time			N-3 PUFA % kcals			Time X N-3 PUFA % kcals		
	Coeff (SE)	Z	P > Z (CI)	Coeff (SE)	Z	P > Z (CI)	Coeff (SE)	Z	P > Z (CI)
MMStot	0.079 (0.035)	2.27	0.023 (0.011,0.147)	0.185 (0.106)	1.75	0.080 (-0.022,0.392)	-0.047 (0.026)	-1.83	0.067 (-0.097,0.003)
CVLtca	-1.127 (0.122)	-9.25	<0.001 (-1.366,-0.888)	-0.155 (0.358)	-0.43	0.665 (-0.857,0.547)	0.117 (0.083)	1.41	0.159 (-0.047,0.279)
CVLfrl	-0.313 (0.058)	-5.38	<0.001 (-0.427,-0.199)	-0.108 (0.169)	-0.64	0.521 (-0.439,0.222)	0.054 (0.039)	1.37	0.171 (-0.023,0.130)
BVRTot	-0.012 (0.085)	-0.15	0.884 (-0.180,0.155)	-0.275 (0.267)	-1.03	0.304 (-0.799,0.249)	0.086 (0.59)	1.45	0.146 (-0.030,0.202)
Attention	-0.010 (0.044)	-0.24	0.811 (-0.096,0.075)	0.024 (0.122)	0.20	0.842 (-0.216,0.264)	-0.032 (0.030)	-1.08	0.279 (-0.090,0.026)
Fluency Word	0.142 (0.084)	1.70	0.089 (-0.022,0.306)	-0.296 (0.286)	-1.03	0.301 (-0.857,0.265)	-0.009 (0.058)	0.15	0.882 (-0.106,0.123)
Digit span Fwd	0.035 (0.036)	0.97	0.334 (-0.036,0.106)	0.080 (0.115)	0.69	0.488 (-0.145,0.304)	-0.012 (0.024)	0.48	0.630 (-0.036,0.060)
Digit span Bck	-0.001 (0.036)	-0.02	0.983 (-0.072,0.071)	-0.036 (0.111)	-0.33	0.744 (-0.253,0.181)	-0.011 (0.025)	0.44	0.661 (-0.038,0.059)
Clock command	-0.020 (0.027)	-0.73	0.464 (-0.072,0.033)	0.109 (0.068)	1.60	0.109 (-0.024,0.241)	-0.004 (0.019)	-0.19	0.846 (-0.040,0.033)
TrailsAtest Sec	0.941 (0.847)	1.11	0.266 (-0.719,2.601)	0.027 (1.978)	0.01	0.989 (-3.851,3.904)	-0.375 (0.604)	-0.62	0.534 (-1.558,0.808)
TrailsBtest Sec	-5.106 (2.184)	-2.34	0.019 (-9.386,-0.826)	3.236 (7.336)	0.44	0.659 (-11.142,17.613)	-0.357 (1.505)	-0.24	0.812 (-3.308,2.593)

Abbreviations: MMStot, Mini-Mental State Exam total score; CVLtca, California Verbal Learning test total correct List A immediate recall; CVLfrl, California Verbal Learning test # correct List A long-delay free recall; BVRTot, Benton Visual Retention test total errors; Attention, Brief test of Attention total correct; Fluency Word, Animal Fluency total words; Digit span Fwd, Wechsler Adult Intelligence Scale- Revised Digit Span Forward total score; Digit span Bck, Wechsler Adult Intelligence Scale- Revised Digit Span Backward total score; Clock command total score; TrailsAtest Sec, Trails A test seconds; TrailsBtest Sec, Trails B test seconds.

Table 3B: Cognitive Change Wave 1 to Wave 3 for n-3HUFA %kcals

Cognitive test	Time			N-3 HUFA % kcals			Time X N-3 HUFA % kcals		
	Coeff (SE)	Z	P > Z (CI)	Coeff (SE)	Z	P > Z (CI)	Coeff (SE)	Z	P > Z (CI)
MMStot	0.050 (0.030)	1.70	0.089 (-0.008,0.109)	0.271 (0.174)	1.56	0.119 (-0.070,0.611)	-0.064 (0.038)	-1.71	0.087 (-0.138,0.009)
CVLtca	-1.057 (0.106)	-9.93	<0.001 (-1.266,-0.848)	0.171 (0.622)	0.28	0.783 (-1.048,1.390)	0.171 (0.127)	1.35	0.178 (-0.078,0.419)
CVLfrl	-0.276 (0.051)	-5.37	<0.001 (-0.376,-0.175)	0.082 (0.292)	0.28	0.780 (-0.491,0.654)	0.007 (0.059)	0.13	0.894 (-0.108,0.124)
BVRTot	0.041 (0.074)	0.55	0.581 (-0.104,0.186)	-0.355 (0.471)	-0.75	0.451 (-1.278,0.569)	0.112 (0.093)	1.20	0.229 (-0.071,0.295)
Attention	-0.027 (0.038)	-0.70	0.484 (-0.101, 0.048)	0.227 (0.212)	1.07	0.283 (-0.188,0.641)	-0.085 (0.045)	-1.86	0.063 (-0.174,0.004)
Fluency Word	0.153 (0.073)	2.11	0.035 (-0.011,0.296)	-0.107 (0.506)	-0.21	0.832 (-1.099,0.884)	-0.064 (0.091)	-0.70	0.482 (-0.243,0.115)
Digit span Fwd	0.046 (0.032)	1.46	0.145 (-0.016,0.108)	0.168 (0.202)	0.83	0.405 (-0.228,0.564)	-0.032 (0.038)	-0.83	0.407 (-0.107,0.043)
Digit span Bck	0.005 (0.032)	0.16	0.869 (-0.057,0.068)	-0.069 (0.195)	-0.36	0.721 (-0.451,0.312)	0.023 (0.039)	0.60	0.549 (-0.053,0.099)
Clock command	-0.022 (0.023)	-0.96	0.335 (-0.068,0.023)	0.130 (0.121)	1.08	0.282 (-0.107,0.366)	0.004 (0.030)	0.13	0.894 (-0.054,0.062)
TrailsAtest Sec	0.663 (0.732)	0.90	0.366 (-0.773,2.098)	-2.605 (3.486)	-0.75	0.455 (-9.438,4.228)	0.152 (0.950)	0.16	0.872 (-1.709,2.014)
TrailsBtest Sec	-5.440 (1.902)	-2.86	0.004 (-9.168,-1.712)	5.410 (12.947)	0.42	0.676 (-19.964,30.786)	0.958 (2.321)	0.41	0.680 (-3.590,5.507)

Abbreviations: MMStot, Mini-Mental State Exam total score; CVLtca, California Verbal Learning test total correct List A immediate recall; CVLfrl, California Verbal Learning test # correct List A long-delay free recall; BVRTot, Benton Visual Retention test total errors; Attention, Brief test of Attention total correct; Fluency Word, Animal Fluency total words; Digit span Fwd, Wechsler Adult Intelligence Scale- Revised Digit Span Forward total score; Digit span Bck, Wechsler Adult Intelligence Scale- Revised Digit Span Backward total score; Clock command total score; TrailsAtest Sec, Trails A test seconds; TrailsBtest Sec, Trails B test seconds.

Table 3C: Cognitive Change Wave 1 to Wave 3 for n-3HUFA/n-6PUFA

Cognitive test	Time			Ratio n-3HUFA/n-6PUFA			Time X n-3HUFA/n-6PUFA		
	Coeff (SE)	Z	P > Z (CI)	Coeff (SE)	Z	P > Z (CI)	Coeff (SE)	Z	P > Z (CI)
MMStot	0.050 (0.027)	1.68	0.093 (-0.008,0.108)	1.249 (0.666)	1.87	0.061 (-0.057,2.555)	-0.257 (0.134)	-1.92	0.055 (-0.519,0.006)
CVLtca	-1.052 (0.106)	-9.89	<0.001 (-1.261,-0.844)	0.846 (2.342)	0.36	0.718 (-3.744,5.436)	0.480 (0.442)	1.09	0.278 (-0.387,1.346)
CVLfrl	-0.274 (0.051)	-5.35	<0.001 (-0.375,-0.174)	0.638 (1.098)	0.58	0.561 (-1.515,2.790)	-0.028 (0.207)	-0.14	0.892 (-0.433,0.377)
BVRTot	0.041 (0.074)	0.55	0.580 (-0.104,0.186)	-2.177 (1.806)	-1.20	0.228 (-5.717,1.364)	0.522 (0.327)	1.60	0.110 (-0.118,1.163)
Attention	-0.029 (0.038)	-0.76	0.450 (-0.103,0.046)	0.941 (0.801)	1.18	0.240 (-0.628,2.510)	-0.278 (0.160)	-1.74	0.082 (-0.592,0.035)
Fluency Word	0.152 (0.073)	2.10	0.036 (-0.010,0.295)	0.399 (1.939)	0.21	0.837 (-3.402,4.120)	-0.235 (0.323)	-0.73	0.465 (-0.868,0.397)
Digit span Fwd	0.044 (0.032)	1.41	0.157 (-0.017,0.107)	0.534 (0.774)	0.69	0.490 (-0.983,2.050)	-0.065 (0.135)	-0.48	0.632 (-0.329,0.200)
Digit span Bck	0.005 (0.032)	0.17	0.869 (-0.057,0.068)	0.191 (0.745)	0.26	0.797 (-1.269,1.652)	0.090 (0.136)	0.66	0.510 (-0.177,0.356)
Clock command	-0.023 (0.023)	-0.99	0.322 (-0.068,0.022)	0.525 (0.462)	1.14	0.256 (-0.381,1.430)	0.043 (0.106)	0.40	0.686 (-0.165,0.251)
TrailsAtest Sec	0.667 (0.731)	0.91	0.362 (-0.767,2.100)	-4.749 (13.339)	-0.36	0.722 (-30.893,21.395)	0.465 (3.403)	0.14	0.891 (-6.205,7.135)
TrailsBtest Sec	-5.409 (1.899)	-2.85	0.004 (-9.132,-1.687)	4.602 (49.474)	-0.09	0.926 (-92.364,101.569)	2.435 (8.093)	0.30	0.763 (-13.427,18.298)

Abbreviations: MMStot, Mini-Mental State Exam total score; CVLtca, California Verbal Learning test total correct List A immediate recall; CVLfrl, California Verbal Learning test # correct List A long-delay free recall; BVRTot, Benton Visual Retention test total errors; Attention, Brief test of Attention total correct; Fluency Word, Animal Fluency total words; Digit span Fwd, Wechsler Adult Intelligence Scale- Revised Digit Span Forward total score; Digit span Bck, Wechsler Adult Intelligence Scale- Revised Digit Span Backward total score; Clock command total score; TrailsAtest Sec, Trails A test seconds; TrailsBtest Sec, Trails B test seconds.

Table 3D: Cognitive Change Wave 1 to Wave 3 for n-3PUFA/n-6PUFA. Significant for MMStot

Cognitive test	Time			Ratio n-3/n-6PUFA			Time X n-3PUFA/n-6PUFA		
	Coeff (SE)	Z	P > Z (CI)	Coeff (SE)	Z	P > Z (CI)	Coeff (SE)	Z	P > Z (CI)
MMStot	0.076 (0.033)	2.30	0.022 (0.011,0.141)	1.058 (0.600)	1.76	0.078 (-0.118,2.234)	-0.255 (0.125)	-2.03	**0.042** (-0.500,-0.009)
CVLtca	-1.113 (0.118)	-9.44	<0.001 (-1.344,-0.882)	0.090 (2.124)	0.04	0.966 (-4.072,4.252)	0.566 (0.417)	1.36	0.175 (-0.251,1.383)
CVLfrl	-0.280 (0.056)	-4.98	<0.001 (-0.391.-0.170)	0.390 (0.998)	0.39	0.696 (-1.566,2.346)	0.045 (0.195)	0.23	0.820 -0.338,0.427)
BVRTot	-0.018 (0.082)	-0.21	0.831 (-0.179,0.144)	-1.724 (1.629)	-1.06	0.290 (-4.918,1.469)	0.558 (0.307)	1.82	0.069 (-0.043.1.160)
Attention	-0.006 (0.042)	-0.15	0.881 (-0.089,0.077)	0.821 (0.729)	1.13	0.260 (-0.608,2.249)	-0.225 (0.151)	-1.49	0.135 (-0.521,0.070)
Fluency Word	0.182 (0.081)	2.24	0.025 (0.022,0.341)	0.411 (1.745)	0.24	0.814 (-3.010,3.832)	-0.274 (0.303)	-0.90	0.366 (-0.867,0.320)
Digit span Fwd	0.049 (0.035)	1.38	0.166 (-0.020,0.118)	0.413 (0.697)	0.59	0.554 (-0.954,1.780)	-0.042 (0.127)	-0.33	0.742 (-0.291,0.207)
Digit span Bck	-0.002 (0.035)	-0.05	0.960 (-0.071,0.068)	0.132 (0.672)	0.20	0.844 (-1.184,1.449)	0.071 (0.128)	0.56	0.576 (-0.179,0.322)
Clock command	-0.022 (0.026)	-0.83	0.404 (-0.073,0.029)	0.560 (0.415)	1.35	0.178 (-0.255,1.374)	-0.005 (0.099)	-0.05	0.963 (-0.198,0.189)
TrailsAtest Se	0.613 (0.822)	0.75	0.456 (-0.998,2.224)	-5.269 (12.039)	-0.44	0.662 (-28.866,18.328)	0.512 (3.167)	0.16	0.872 (-5.696,6.719)
TrailsBtest Sec	-5.551 (2.112)	-2.63	0.009 (-9.691,-1.411)	18.110 (44.648)	0.41	0.685 (-69.398,105.618)	1.491 (7.645)	0.19	0.845 (-13.493,16.475)

Abbreviations: MMStot, Mini-Mental State Exam total score; CVLtca, California Verbal Learning test total correct List A immediate recall; CVLfrl, California Verbal Learning test # correct List A long-delay free recall; BVRTot, Benton Visual Retention test total errors; Attention, Brief test of Attention total correct; Fluency Word, Animal Fluency total words; Digit span Fwd, Wechsler Adult Intelligence Scale- Revised Digit Span Forward total score; Digit span Bck, Wechsler Adult Intelligence Scale- Revised Digit Span Backward total score; Clock command total score; TrailsAtest Sec, Trails A test seconds; TrailsBtest Sec, Trails B test seconds.

Chapter 5

DISCUSSION

5.1 General Discussion

Significant differences were noted in the cognitive performance for visual and verbal memory tests between Wave 1 and Wave 3 indicating that there was some cognitive decline detected during this time interval. Nevertheless, there were no significant relationships between outcomes of cognitive change for 11 cognitive tests and the exposures of n-3 PUFA, n-3 HUFA and their ratios, except for one. Higher ratios of n-3 PUFA/n-6 PUFA were associated with slower rates of change in the Mini-Mental State Examination total score from Wave 1 to Wave 3 (b= -0.255, SE 0.125; P>Z 0.042, 95% CI -0.500, -0.009). The finding that only one significant relationship was found across four models for 11 outcomes, suggests that this one instance of significance could be the result of a type-1 error, a statistical artifact, rather than support for the study hypothesis.

There are several possible reasons for the lack of significant relationships between exposures and cognitive change in this study. First, it is possible that the total intake of n-3 PUFA was too low for a significant association to be seen. The Acceptable Macronutrient distribution range (AMDR) for the essential short chain n-3 PUFA, ALA is 0.6-1.2% E (Institute of Medicine, 2005). The mean intake of n-3 PUFA in this sample for ALA was 0.6309 % E, which is at the lower end of the AMDR, and for the total PUFAs (= ALA+ EPA + DPA + DHA) was 0.7384 % E (SE = 0.0173; 95% CI, 0.7045-0.7722). Although there is no dietary reference intake (DRI) for the long-chain n-3HUFAs, the Institute of Medicine (IOM) recommends $\leq$ 10% of total AMDR for ALA as EPA and/or DHA, which is lower than the

recommendation of many other global organizations (Kris-Etherton, Grieger, & Etherton, 2009). Based on these values, the mean intake of n-3 fatty acids in this study sample was at the lower limits of the AMDR, and may have been too small to demonstrate a significant association with cognitive decline.

Regarding the ratios of n-3/n-6 PUFA, there are no consistent recommendations. The suggested guideline by the Institute of Medicine (IOM) for n-3 ALA relative to n-6 LA is 1:5-10. Despite this, studies with constant n-3 ALA intakes ~ 0.5 -1% E showed that low intakes of n-6 LA <2% E were associated with higher biomarkers of the n-3HUFAs, EPA and DHA, compared to higher intake of LA of 4-8% E. These findings suggest that ratios closer to 1:2 may be most beneficial (Liou et al., 2007; Wood et al., 2014, 2015).

Another explanation is that there may have been residual confounding variables not accounted for in our study. In human studies, it is very difficult to separate intake of one dietary component from other dietary confounding variables, such as dietary patterns, nutrients, or related health habits. With respect to dietary patterns, many studies support a neuroprotective effect of healthful dietary patterns and adherence to the Mediterranean diet, in particular (Kuczmarski et al., 2014). A recent study of the Mediterranean-Dietary Approach to Systolic Hypertension (MIND) diet, a modification of the Mediterranean and DASH diets, showed higher MIND scores were associated with slower decline in multiple cognitive domains in older adults over an average of 4.7 years (Morris, Tangney, Wang, Sacks, Barnes, et al., 2015). The difference in rate of decline between the top and bottom tertiles equated to being 7.5 years younger. This group also showed the MIND score was associated with lower risk of Alzheimer's disease, to a greater extent than either the Mediterranean or

DASH diets (Morris, Tangney, Wang, Sacks, Bennett, et al., 2015). Unfortunately, the African American and White participants of HANDLS consumed similar dietary patterns, all reflecting an unhealthful Western diet (Kuczmarski et al., 2015).

Regarding other nutritional factors, interactions of n-3 fatty acids with nutrients such as the B vitamins, alpha-lipoic acid and polyphenols have been observed. A Vitamin B supplementation (folic acid, B6 and B12) trial demonstrated a beneficial effect of high baseline serum n-3 fatty acids >590 µmol/L, with slower rates of cognitive decline and reduced rates of brain atrophy, but only in the B vitamin supplementation group, not in controls (Jerneren et al., 2015; Oulhaj, Jernerén, Refsum, Smith, & de Jager, 2016). Another trial of n-3 supplementation, with or without alpha lipoic acid compared to placebo, measured cognitive and functional performance in 39 subjects with Alzheimer's disease over 12 months (Shinto et al., 2014). Compared to placebo controls, both n-3 groups showed less decline in Activities of Daily Living, while only the n-3 + alpha lipoic acid group showed less decline in Mini-Mental State Examination.

Consumption of plant polyphenols, such as those in grapes and red wine, stimulates endogenous synthesis of 'marine' n-3 HUFAs from their plant substrate ALA in rats, and increases plasma n-3 HUFA levels in humans (de Lorgeril, Salen, Martin, Boucher, & de Leiris, 2008; Giuseppe et al., 2009; Toufektsian, Salen, Tonelli, & Lorgeril, 2011). Large amounts of ALA and the polyphenol anthocyanins, also low quantities of n-6 fats, with balanced n-3/n-6 ratio similar to the ancient diet are found in the traditional Mediterranean diet, which at least partially accounts for the protection of this diet against cognitive impairment (de Lorgeril & Salen, 2012; Simopoulos, 2009). Although the current study excluded low nutrient blood levels of

Vitamin B12 and folate, the models did not adjust for them. It is possible that there were nutrient interactions not measured in this study, or that may not yet have been elucidated in the literature.

In addition to the diet, more recent evidence supports a potential benefit of combining multiple lifestyle, psychosocial, and other environmental factors on cognitive decline and dementia. One trial among 2,654 at-risk, non-demented elderly adults applied multiple interventions of diet, exercise, vascular risk monitoring and cognitive training (treatment group) or general health advice (controls) in a randomized controlled design (Ngandu et al., 2015). After two years, the treatment group showed improvements in comprehensive neuropsychological test battery Z score, executive function, and processing speed that were significantly greater than controls, by 25-150%.

Another multimodality trial of n-3 fatty acid supplementation (2200 mg long-chain), aerobic exercise and cognitive stimulation (target intervention) or n-3 supplementation and non-aerobic exercise (controls) studied 22 MCI patients aged 60-80 years, (Köbe et al., 2016). After six-months, while there were no differences in cognitive performance, significant differences were seen in Alzheimer's-related brain gray matter regions. The control group had decreased frontal, parietal and cingulate gyrus gray matter volume, while those structures in the target group were preserved or even increased.

A novel therapeutic multimodality pilot program to enhance neurodegeneration (MEND) was administered over 5-24 months among ten patients with subjective cognitive impairment, MCI and Alzheimer's disease (nine ApoE4+), some of whom were high level professionals and business owners whose impairments forced them to

stop working. Personalized dietary, metabolic and hormonal optimization, stress reduction, supplements, and a brain-training program were used. Not only were subjective improvements reported by the patients, their spouses and co-workers, but in all cases improvements were demonstrated on neuropsychological testing, and in one case a striking hippocampal volume increase was observed, to a degree not previously reported (Bredesen, 2014; Bredesen et al., 2016). Therefore, a multifactorial approach will likely be more effective than a single intervention, to effectively prevent and potentially reverse cognitive decline and dementia, including Alzheimer's disease.

5.2 Strengths and Limitations

This study had several strengths. First, there was robust baseline dietary data which included two 24-hour dietary recalls. Furthermore, data were collected and analyzed for six n-3 and n-6 fatty acids, so that n-3/n-6 ratios could be assessed and HUFAs could be analyzed separately from PUFAs. Another strength is that the large study sample was an under-represented group of biracial, socioeconomically diverse, community-dwelling working aged adults, reflective of the urban Baltimore city population. Finally, a large cognitive test battery was administered for assessment of multiple cognitive domains.

Several limitations can be noted. First, our study did not assess polymorphisms such as presence of the ApoEε4 allele, a known genetic risk factor for AD, or the desaturase (FADS1 and FADS2) and elongase (ELOVL1 and ELOVL2) genes. The presence of the apolipoprotein ApoEε4 allele has been shown to alter DHA metabolism. A controlled study demonstrated disturbance of [13-C]-n3DHA metabolism among carriers (E4+) of the ApoEε4 allele (Chouinard-Watkins et al., 2013). Over a 28-day supplementation trial of 40 mg [13-C]-n3DHA, carriers of the

ApoEε4 allele had 31% lower mean plasma [13-C]-n3DHA, higher beta-oxidation, and 77% lower whole-body half-life of [13-C]-n3DHA than non-carriers.

Although the presence of the ApoEε4 allele is not controlled for in all studies, even in studies that do so, there may be inconsistent results. For example, some studies showed that greater fish consumption (Barberger-Gateau et al., 2007) or higher levels of erythrocyte n-3 PUFA were associated with significant cognitive benefit, reduced risk of dementia and Alzheimer's disease only in non-carriers of the ApoEε4 allele (Huang et al., 2005; Whalley et al., 2008). On the other hand, others found higher plasma DHA levels, or higher intakes of seafood and HUFAs were associated with slower rates of cognitive decline over multiple domains, but only in the ApoEε4 carriers (Samieri et al., 2011; van de Rest et al., 2016).

Other genetic variations, such as fatty acid desaturase (FADS) polymorphisms, can impact metabolism of LA and ARA (Mathias et al., 2011). The homozygous GG alleles for fatty acid desaturase Δ5 (FADS-1) are more efficient than the GT or TT polymorphism in conversion of the n-6 intermediate dihomo-gamma-linoleic acid (DGLA) to ARA, leading to higher levels of the ARA, and potentially the ARA-derived eicosanoids. The frequency for this more efficient homozygous GG polymorphism varies significantly depending on race and origin, having been found to in 97.5% of continental Africans, 79-82 % of African Americans, and only 42-45% of European Americans (Chilton et al., 2014; Mathias et al., 2011). Genetic variables are key factors in PUFA physiology that should be included in the research.

In this study, no serum or erythrocyte n-3 and n-6 fatty acid bioassays were analyzed. An inherent weakness of nutritional epidemiologic studies that use dietary surveys for n-3 PUFA intake is limited reliability of dietary intake data, as well as

questionable correlation of dietary intakes with serum and erythrocyte biomarkers. While biological markers are considered more accurate assessments of fatty acid status than dietary data, even studies that measured blood biomarkers such as erythrocyte membrane n-3 PUFA were inconsistent. Some showed benefits of increased plasma or red blood cell n-3 HUFA levels on decreased risk for dementia (Phillips et al., 2012; Tan et al., 2012) or better cognitive performance (D'Ascoli et al., 2016), while others observed an inverse association of cognitive function with fish intake, but not with erythrocyte membrane levels (Danthiir et al., 2014). It is possible that even the most reliable dietary recalls and plasma markers may not be accurate indicators of long-term intakes, age-related alterations in PUFA homeostasis which may be independent of intakes, and even more importantly, they may not correlate with brain and total body DHA levels (Cunnane et al., 2013; Hennebelle et al., 2013; Otsuka, Kato, Imai, Ando, & Shimokata, 2013).

Another limitation is that no baseline supplementation data were available. Therefore, we could not account for the potential influence of n-3 fish oil and other supplement use on the cognitive outcomes. In addition, although adjustments were not made for known cardiovascular disease, several specific cardiovascular risk factors such as cigarette status, systolic BP, and BMI were included in the model.

Finally, there was no baseline physical activity data in the HANDLS study. Physical activity and n-3 fatty acids have each been independently associated with higher cognitive function, decreased risk of cognitive impairment later in life, and greater volume of the prefrontal cortex and hippocampal gray matter (Bherer, Erickson, & Liu-Ambrose, 2013; Erickson et al., 2011; Leckie et al., 2014; Middleton, Barnes, Lui, & Yaffe, 2010). Even more interestingly, an interaction between physical

activity and the ratio of n-6 to n-3 fatty acid serum levels has been shown, in which higher levels of n-3 mitigated the detrimental effects of lower physical activity levels (Leckie et al., 2014).

5.3 Conclusion

Epidemiological and interventional evidence supporting the neuroprotective benefits of n-3 fatty acids, and more specifically, the absolute and relative status of n-3 HUFAs, is encouraging, but inconsistent. In addition, mechanistic evidence is growing for an essential role of n-3 fatty acids in brain structure and function. To optimize n-3 HUFA status, it is important to not only increase dietary n-3 HUFA consumption, but to also decrease intake of n-6 fatty acids. Dietary sources rich in n-3 HUFAs include fatty fish such as salmon, mackerel, herring, lake trout and sardines, high n-3 eggs, algal oil, as well as krill and fish oil supplements. The Dietary Guidelines for Americans, 2010 recommends eating 8 ounces of oily fish per week (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010). Vegetable oils with the highest content of n-6 fatty acids, including soybean, safflower, sunflower, corn, and cottonseed oils should be limited. Fats low in n-6 PUFA content, such as olive oil, coconut oil, n-3 rich flaxseed oil and butter/ghee could be used as replacements. Decreased consumption of ARA-rich meats would lower n-6 intakes. These measures could help shift the balance toward a more optimal n-3 level and n-3/n-6 HUFA ratio.

Further research should focus on the competitive dynamics of the n-3 and n-6 fatty acids, the determination of an optimal balance, and how that balance relates to normal brain structure and function, as well as to prevention and delay of neuropathological processes leading to cognitive decline and dementia.

Although improving n-3 absolute and relative fatty acid status should be one component of a program for optimal brain health and cognitive function, it is unlikely that modification of one isolated nutritional component will have a far-reaching impact on prevention or treatment of cognitive decline and its potential endpoints of dementia and Alzheimer's disease, without inclusion of more wide-ranging interventions in diet, and multiple other lifestyle arenas, not to mention changes in the food industry. As with other chronic disorders such as cardiovascular disease or cancer, cognitive decline is a complex interaction of multifactorial processes (Kiecolt-Glaser, 2010). The conventional paradigm of "one disease-one cure" has not worked well for chronic illness. If health professionals intend to effectively prevent, delay and potentially reverse cognitive decline and dementia, including Alzheimer's disease, we must begin to think more expansively. Stemming this public health tsunami, the suffering of countless victims and their families, and the strain on our healthcare system and economy will require a more comprehensive, multifactorial, and even an individually tailored approach, that considers genetic as well as nutritional, physical, psychosocial and cognitive factors.

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Appendix A

IRB APPROVALS

A.1 HANDLS IRB Approved Consent Wave 1

IRB number: 2003-314 **Clinical Site IC Version: 08/22/2005**
Project Title: **Healthy Aging in Neighborhoods of Diversity across the Life Span**
Principal Investigator: MK Evans & AB Zonderman **Institution:** National Institute on Aging, NIH

MedStar Research Institute

Informed Consent for Clinical Research – HANDLS Phase 1

SITE: Mobile Medical Research Vehicles (MRVs) – 13 neighborhoods in Baltimore City

PRINCIPAL INVESTIGATOR: Michele K. Evans, M.D. & Alan B. Zonderman, Ph.D.

Co-INVESTIGATORS: D.R. Abernethy, M. Brock, N. Ejiogu, K. Foster, M.C. Gibbons, J. Kelley-Moore, M.H. Kitner-Triolo, M.T. Fanelli Kuczmarski, T.A. LaVeist, J. Lepkowski, S. Ling, E. Nagababu, S. Najjar, N.R. Powe, J.M.Rifkind, J.F. Thayer, A. Trzeciak

INTRODUCTION

We invite you to take part in an observational research study called Healthy Aging in Neighborhoods of Diversity across the Life Span. You were selected as a possible participant in this study because we are looking for residents from your neighborhood between the ages of 30 and 64 years old. Please take your time to read this form, ask any questions you may have and make your decision. We encourage you to discuss your decision with your family and friends.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to learn about changes in health over time. We want to study as many people in different neighborhoods as we can. Our goal is to study health change as people grow older. We plan to do this by studying the same people over many years. This gives us the information we want about how peoples' bodies change over time. We also want to study why some people are healthier than others as they get older. We want to discover if we can predict the causes of good health with aging. If we can find the causes of good health, then we might find the cures for some of the diseases related to aging. This is an observational study where we will follow you over the next twenty years to see how you age. This will help us learn about the natural course of diseases like heart disease, Alzheimer's disease, hypertension, diabetes and stroke. We are trying to understand why some Americans have higher rates of certain diseases and more severe diseases than other Americans. This research is being done so that we can discover better ways to prevent and treat disease.

WHAT ELSE SHOULD I KNOW ABOUT THIS RESEARCH STUDY?

It is important that you read and understand several points that apply to all who take part in our studies:

- Taking part in the study is entirely voluntary and refusal to participate will not affect any rights or benefits you normally have;
- You may or may not benefit from taking part in the study, but knowledge may be gained from your participation that may help others; and
- You may stop being in the study at any time without any penalty or losing any of the benefits you would have normally received.

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The nature of the study, the benefits, risks, discomforts and other information about the study are discussed further below. If any new information is learned, at any time during the research, which might affect your participation in the study, we will tell you. We urge you to ask any questions you have about this study with the staff members who explain it to you and with your own advisors before agreeing to participate.

WHO IS IN CHARGE OF THIS STUDY?

The research is being conducted and sponsored by the National Institute on Aging with Michele K. Evans, M.D. and Alan B. Zonderman, Ph.D. as the primary investigators.

WHO CANNOT PARTICIPATE IN THIS STUDY?

You cannot be in this study if any of the following apply to you;

If you:

- Do not have a valid picture ID
- Are unable to give informed consent
- Are under 30 years old
- Are older than 64 years old
- Are pregnant
- Are currently undergoing cancer treatment (chemotherapy or radiation)
- Have undergone cancer treatment (chemotherapy or radiation) within the last 6 months

WHAT IF I AM PRESENTLY PARTICIPATING IN ANOTHER RESEARCH STUDY?

Are you presently participating in any other research studies? Yes ☐ No ☐

If yes, please state which study(ies):_____

While participating in this study, you should not take part in any other research project without approval from the people in charge of each study. This is to protect you from possible injury arising from such things as extra blood drawing, extra x-rays, interaction of research drugs, or similar hazards.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About **4000** people will take part in this study, **around 335** from your neighborhood.

WHAT HAPPENS IF I AGREE TO BE IN THE STUDY?

The study data will be collected in two parts. This is a consent form for the first part. You are required to give your consent for both parts.

This first part of the study consists of a household interview. This interview includes questions about your age, occupation, and neighborhood. We also want to know about your physical activities, use of dental and health services,

and stress that you might experience and how you deal with it. We will also ask you to remember all of the food that you ate in the past day. We will discuss the way the household interview will be conducted below.

In the second part of the study, you will spend a day at our Mobile Medical Research Vehicles (MRVs). While you are there, we will ask you for additional information and we will do additional tests. You will be asked about your medical history and you will receive a physical examination. We will ask what you ate during the last 24 hours. You will receive memory testing. We will also measure your emotions and heart rate changes, muscle strength, bone density and test for hardening of the arteries. We will also take blood, tissue and urine samples. You will be offered a test for HIV. If you decide to have the test, you will be asked to sign a separate consent form that explains the HIV testing procedures for the HANDLS study. You will also be asked to give a DNA sample by using a method called Buccal Mucosa smear. Before you agree to give the DNA sample you will be required to sign a separate consent that explains the procedures and risks of providing DNA samples. More details about the tests for the second part of the study are described in the attached **Consent Form Booklet**.

This first part of the study will take place in your home. We will ask you to answer questions about you and your physical activity, use of dental and health services, stress and how you deal with it and a few questions about your neighborhood. We will also ask you to complete a dietary recall questionnaire that asks you to remember what you had to eat and drink in the last 24 hours. We will use pictures to help you give us information about how much food and drink you had in the last 24 hours. We expect this household interview to take about 90 minutes.

All of the questionnaires collect information about our research. They are not designed to improve your health at this time. We perform these questionnaires free of charge. You may participate in both of the questionnaires, but you do not have to. You may stop any questionnaire after it starts. This will not affect your right to participate in this study. This is a longitudinal study. Our Mobile Medical Research Vehicles will return to your neighborhood every three years and we will ask you again at that time to participate in this study.

HOW LONG WILL I BE IN THE STUDY?

We think you will be in the study for the next 20 years because this is a longitudinal study that follows your health over time as you age. This is a study that provides long-term follow up. The study doctor or the National Institute on Aging may stop your participation in this study at any time without your consent. Any information (data) or blood collected until that point in time would remain part of the study. You can stop participating at any time. However, if you decide to stop participating in the study, we ask you to talk to the researchers first.

WHAT ARE THE RISKS AND SIDE EFFECTS OF THIS STUDY?

If you decide to participate in this study, you should know there may be risks. The risks for this study are minimal. The descriptions of the tests given on the Mobile Medical Research Vehicles include any risks and other possible side effects. They are also explained in the **Consent Booklet** under the Assessment of Risks section. For more information about risks and side effects, you should call the Principal Investigator, Michele K. Evans, M.D. at 410-558-8573.

As part of this study, you will be asked to sign a separate consent form to be in the part of this study involving genetic testing. Risks of genetic testing include the misuse of personal, genetic information. Although rare, misuse of such information has caused problems for persons related to employment, life, or health insurance benefits and right. There is a risk that being in a genetics study can cause psychological distress or tension with other family members. Although

there can be no absolute guarantees, every reasonable effort will be made to keep your personally identifiable information secret so that there will be no misuse. Even when the information is kept secret, if you are asked if you have ever been tested for a genetic disorder, answering "yes" could cause benefits to be denied or could cause other problems including discrimination.

ARE THERE ANY BENEFITS TO TAKING PART IN THE STUDY?

This study is not designed to provide direct benefits to any participants. If you agree to take part in this study, there may or may not be direct medical benefits to you. We hope the information learned from this study will benefit others in the future. There is no charge for any of the testing described. You may benefit by learning more about your health, or possibly from learning that you have a condition or problem. You will receive a Participant Report Package in the mail, with results of your visit to the MRVs. If the study doctor discovers any condition or problem, the information will be provided to you and your doctor, if you authorize it. To authorize the reporting of results to your physician you will need to sign a form called "Release of Medical Information". You will be asked to sign this form only if you want us to communicate with your physician. The study doctors do not provide medical treatment. The information gained from this research may benefit others in the future.

WHAT OTHER OPTIONS ARE THERE?

There are no other options associated with your participation in this study. You may choose either to participate or not to participate in this research. Taking part in this study is entirely voluntary. You may choose to withdraw from the study at any time.

WHAT ABOUT CONFIDENTIALITY?

Your personal health information (PHI) will be kept private to the extent allowed by law. You will not be identified by name in any publications resulting from this study. You will be asked to sign a separate form that will give permission to the investigator, the sponsor, and certain other people, agencies or entities to look at and review the records related to this study including your personal health information and the information discovered during this study. If you do not wish to sign this permission form you will not be allowed to participate in this study.

Personal Health Information (PHI) is stored in secure databases. These databases are password protected and maintained on a secure NIA/NIH system with access limited to authorized NIA staff. All NIA staff that has access to these databases has the proper training on patient confidentiality as well as the required Human Subject Protection Training. The system is administered using the security policies and regulations required by the National Institutes of Health consistent with the Health and Human Services Privacy Rule and HIPAA.

Organizations that may request, inspect and/or copy your research and medical records for quality assurance and data analysis include groups such as: the National Institute on Aging, Office of Human Research Protection, MedStar Research Institute, Institutional Review Board (IRB), Coda and Westat.

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this certificate the researchers cannot be forced to disclose information that may identify you, even by court subpoena, in any federal, state, or local civil, criminal, administrative, legislative or other proceedings. The researchers will use the certificate to resist any demands for information that would identify you, except as explained below.

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The Certificate cannot be used to resist a demand for information from personnel of the U.S. Department of Health and Human Services that is used for auditing or program evaluation or for information that must be disclosed in order to meet federal regulations.

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researcher may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent researcher from disclosing voluntarily, without your consent, information that would identify you as a participant in the research project under the following conditions: It does not apply to state requirements to report certain communicable diseases. In addition, the study doctor may be required to report certain cases of abuse, neglect, or suicidal or homicidal intent to the appropriate authorities.

WILL I BE PAID FOR PARTICIPATING IN THIS STUDY?

You will be paid $100 for participating in this study. You will receive your payment in the form of an ATM debit card at the end of the MRV visit. **If you are unable to complete all of the tests you will receive a portion of the payment.** The ATM card will be activated before you leave the vehicle. You will be able to take the card to an ATM machine in your neighborhood to withdraw your payment. We will provide round-trip transportation from your home to our mobile testing center if you want it. We will serve a box breakfast and box lunch if you are participating in tests during mid-day. We will do our best to meet your dietary needs if you have any.

WHAT ARE THE COSTS?

You do not have to pay anything to be in this study. However, if taking part in this study leads to procedures or care not included in the study, it may lead to added costs for you or your insurance company. You will not be charged for any tests or procedures that are part of this research study.

WHAT IF I'M INJURED OR BECOME ILL DURING THE STUDY?

We will make every effort to prevent injuries and illness from being in the study. If you have any adverse experience resulting directly from the study, the National Institute on Aging *will* provide or pay for short-term medical care for any injury resulting from participation in research here as long as the costs are not covered by your medical or hospital insurance. You should not expect any one to pay you for pain, worry, lost income, or non-medical care costs that occur from taking part in this research study. No other form of compensation is available for any adverse experience. *The National Institute on Aging, National Institutes of Health, the Federal Government, the MedStar Research Institute, MedStar Health, CODA or Westat* do not have money set aside to repay you in case of injury.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

You have the right to be told about the nature and purpose of the study;
You have the right to be given an explanation of the exactly what will be done in the study and given a description of potential risks, discomforts, or benefits that can reasonably be expected;

You have the right to be informed of any appropriate alternatives to the study, including, if appropriate, any drugs or devices that might help you, along with their potential risks, discomforts and benefits;
You have the right to ask any questions you may have about the study;
You have the right to decide whether or not to be in the study without anyone misleading or deceiving you; and
You have the right to receive a copy of this consent form.

By signing this form, you will not give up any legal rights you may have as a research participant. You may choose not to take part in or leave the study at any time. If you choose to not take part in or to leave the study, you will not lose any of the benefits you would have received normally. We will tell you about new information that may affect your health, welfare, or willingness to be in this study.

WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, contact the investigator, Michele K. Evans, M.D., at (410)-558-8573. For medical assistance during the evening or on weekends, call the NIA Security Office at (410) 558-8119 and request that they contact the NIA Physician-on-Call.

If you are having a medical emergency, you should call 911 or go directly to the nearest emergency room.

If you are injured as a result of being in a study, or think you have not been treated fairly, please contact the NIA Clinical Director or Deputy Clinical Director at (410) 350-3922.

For questions about your rights as a research participant, you can call or write the following:

NIA Clinical Director
3001 S. Hanover Street, 5th Floor
Baltimore, MD 21225
Phone (410) 350-3922

NIA Clinical Research Protocol Office
3001 S. Hanover Street, Room 539
Baltimore, MD 21225
Phone: (410) 350-3947
Fax: (410) 350-3979.

MedStar Research Institute
Office of Regulatory Affairs
6495 New Hampshire Avenue, Suite 201
Hyattsville, MD 20783
Phone: (301) 560-7339
Toll Free: (800) 793-7175
Fax: (301) 560-7336

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OCT 2 4 2006
Form Revision Date: 05/10/04

SIGNATURES

As a representative of this study, I have explained the purpose, the procedures, the possible benefits and risks that are involved in this research study. Any questions that have been raised have been answered to the individual's satisfaction.

Signature of Person Obtaining Consent — Date of Signature

I, the undersigned have been informed about this study's purpose, procedures, possible benefits and risks, and I have received a copy of this consent. I have been given the opportunity to ask questions before I sign, and I have been told that I can ask other questions at any time. I voluntarily agree to be in this study. I am free to stop being in the study at any time without need to justify my decision and if I stop being in the study I understand it will not in any way affect my future treatment or medical management. I agree to cooperate with Dr. Michele K. Evans, Dr. Alan B. Zonderman, and the research staff and to tell them immediately if I experience any unexpected or unusual symptoms.

Participant's Signature — Date of Signature

Signature of Witness — Date of Signature

Signature of Legally Authorized Representative (When Appropriate) — Date of Signature

Relationship to Participant (When Appropriate) — Date of Signature

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A.2 HANDLS IRB Approved Consent Wave 3

IRB number: 2009-149 **Clinical Site IC Version:** 05/27/2009
Project Title: **Healthy Aging in Neighborhoods of Diversity across the Life Span Wave 3 Phase 1**
Principal Investigator: MK Evans & AB Zonderman **Institution:** National Institute on Aging, NIH

MedStar Research Institute

Informed Consent for Clinical Research
HANDLS Wave 3 - Phase 1 MRV Visit

SITE: Mobile Medical Research Vehicles (MRVs) -- Neighborhoods in Baltimore City

PRINCIPAL INVESTIGATOR: Michele K. Evans, M.D. & Alan B. Zonderman, Ph.D.

Co-INVESTIGATORS: D. E. Arking, J. Coresh, N. Ejiogu, C. Fletcher, M.H. Kitner-Triolo, M.T. F. Kuczmarski, T.A. LaVeist, J. Lepkowski, N.R. Powe, A. Singleton, A. Trzeciak, S. Waldstein, S. Najjar

INTRODUCTION

We are inviting you to take part in the next phase of the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. You may remember you were selected a few years ago to participate in this study because we were looking for residents from your neighborhood between the ages of 30 and 64 years old and because you decided you wanted to take part in the study. It is time for us to return to your neighborhood for the first follow-up examination. You now have an opportunity to decide whether you would like to participate in the next phase of HANDLS. You will notice that some of the tests are the same as the last time we saw you. We have added some different tests and questionnaires that you might not be familiar with. Please take your time to read this form. Be sure to ask any questions you may have before making your decision. We encourage you to discuss your decision with your family and friends.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to learn about changes in health over time in an urban, working and non-working group of African-American and white, men and women residing in Baltimore city. We want to study as many people in different neighborhoods as we can. Our goal is to study health change as people grow older. We plan to do this by studying the same people over many years. This gives us the information we want about how peoples' bodies change over time.

We also want to study why some people are healthier than others as they get older. We want to discover if we can predict the causes of good health with aging. If we can find the causes of good health, then we might find the cures for some of the diseases related to aging. This is a research study where we will follow you over the next twenty years to see how you age. This will help us learn

about the natural course of diseases like heart disease, Alzheimer's disease, high blood pressure, diabetes and stroke. We are trying to understand why some Americans have higher rates of certain diseases and more severe diseases than other Americans. This research is being done so that we can discover better ways to prevent and treat disease.

WHAT ELSE SHOULD I KNOW ABOUT THIS RESEARCH STUDY?

It is important that you read and understand several points that apply to all who take part in our studies:

- Taking part in the study is entirely voluntary and refusal to participate will not affect any rights or benefits you normally have;
- You may or may not benefit from taking part in the study, but knowledge may be gained from your participation that may help others; and
- You may stop being in the study at any time without any penalty or losing any of the benefits you would have normally received.

The nature of the study, the benefits, risks, discomforts and other information about the study are discussed further below. The information is also explained in the informed consent booklet that goes with this consent form. If any new information is learned, at any time during the research, which might affect your participation in the study, we will tell you. It is important for you to ask any questions you have about this study with the staff members who explain it to you and with your own advisors before agreeing to participate.

WHO IS IN CHARGE OF THIS STUDY?

The research is being conducted and sponsored by the National Institute on Aging with Michele K. Evans, M.D. and Alan B. Zonderman, Ph.D. as the primary investigators.

WHO CANNOT PARTICIPATE IN THIS STUDY?

You cannot be in this study if any of the following apply to you;

If you:

- Did not give your consent to be in the HANDLS Wave 1study during the recruitment phase
- Do not have a valid picture ID
- Are unable to give informed consent
- Are pregnant
- Are currently undergoing cancer treatment (chemotherapy or radiation)

- Have undergone cancer treatment (chemotherapy or radiation) within the last 6 months

WHAT IF I AM PRESENTLY PARTICIPATING IN ANOTHER RESEARCH STUDY?

Are you presently participating in any other research studies? Yes ☐ No ☐

If yes, please state which study(ies):_____

While participating in this study, you should not take part in any other research project without approval from the people in charge of each study. This is to protect you from possible injury arising from such things as extra blood drawing, extra x-rays, interaction of research drugs, or similar hazards.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About **3724** people will take part in this study, **around 300** from your neighborhood.

WHAT HAPPENS IF I AGREE TO BE IN THE STUDY?

The HANDLS Wave 3 study data will be collected in two parts. You are required to give your consent for each part of the study. You may also be invited to participate in a third part of HANDLS Wave 3. The third part of HANDLS Wave 3 consists of two additional studies to be conducted at Harbor Hospital and University of Maryland. You will learn more about those studies during this examination visit, if you are eligible to participate.

This is the consent form for the first part of HANDLS Wave 3. You will be required you to spend a day at our Mobile Medical Research Vehicles (MRVs) to have testing. You will be asked provide an update about your medical history since your last examination and you will receive a physical examination. We will ask you to remember all of the food you ate the day before your visit. We will assess your muscle strength and bone density. You will have a test to check the blood flow in your heart and to see if your heart valves are leaking. We will also take blood, tissue and urine samples. You will be offered a test for HIV. If you decide to have the test, you will be asked to sign a separate consent form. It will explain the HIV testing procedures for the HANDLS study. You will also be asked to give a DNA sample by using a method that collects cells from a saliva (spit) sample you provide. Before you agree to give the DNA sample you will be required to sign a separate consent that explains the procedures and risks of providing DNA samples. More details about the specific testing for this part of the study are described below and in the attached **Consent Booklet**.

The second part of this study will be given as a one hour telephone interview. It will be scheduled at the end of your MRV visit. During the telephone interview, you will be asked once again to remember all the food you ate the day before your telephone interview. We will also ask you to complete a questionnaire. You will be asked about activities of daily living, use of health care services, and any income and/or employment changes since your last visit to the MRVs.

The tests involved in this study are described in the attached **Consent Booklet**. All of the tests are performed for the purpose of research and are not designed to improve your health at this time. There are no experimental medications, tests or procedures in this study. We perform these tests free of charge. If, after reading the **Consent Booklet**, there are tests in which you do not wish to participate, please list them on the back of this form.

Below is a table that shows the tests you will be expected to complete. This chart also tells you how long we think it will take each test to be done and in which vehicle it will be given.

Phase 1 – Medical Research Vehicle Examination

Measure or Procedure	Estimated Timing	Location
Consent	20 minutes	MRV2/3
Specimen Collection (Urine, Blood, DNA)	20 minutes	MRV 3
Anthropometrics (height & weight)	5 minutes	MRV 1
Interim Medical History	20 minutes	MRV 1
Interim Physical Exam	20 minutes	MRV 1
Dietary Recall I	30 minutes	MRV 2
Cognition	40 minutes	MRV 2
Physical Performance	15 minutes	MRV 1
Echocardiogram	20 minutes	MRV 1
Questionnaires Section A	35 minutes	MRV 2
Body Composition/Bone Densitometry	30 minutes	MRV 1

You may participate in any of the tests, but you do not have to participate in all of the tests. This will not affect your right to participate in this study. You may stop any test after it starts. If you are unable to complete all of the tests in one visit you may be invited to return to the MRVs to complete your testing.

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This is a long-term study, our Mobile Medical Research Vehicles will be back in your neighborhood every three years and we will ask you again at that time to participate in this study.

HOW LONG WILL I BE IN THE STUDY?

You are being asked to be in the study for the next 20 years. This is a longitudinal study that follows your health over time as you age. This is a study that provides long-term follow up. The study doctor or the National Institute on Aging may stop your participation in this study at any time without your consent. Any information (data) or blood collected until that point in time would remain part of the study. You can stop participating at any time. However, if you decide to stop participating in the study, we ask you to talk to the researchers first.

WHAT ARE THE RISKS AND SIDE EFFECTS OF THIS STUDY?

If you decide to participate in this study, you should know there may be risks. The risks for this study are minimal. The descriptions of the tests given on the Mobile Medical Research Vehicles include any risks and other possible side effects. They are also explained in the **Consent Booklet** under the Assessment of Risks section.

This research study requires a small amount of radiation from the DEXA Scan. It must be noted that this radiation exposure is not needed for your medical care. It is for research purposes only. The total amount of radiation you will receive from this study is from one DEXA scan. The NIH Radiation Safety Committee has reviewed the use of radiation in this research study. It has approved this use as involving minimal risk and needed to obtain the research information desired.

Using the standard way of describing radiation exposure, from one DEXA Scan you will receive an effective dose of less than one thousandth of one rem. By comparison the average person in the United States receives this much radiation every day from natural sources, such as the sun. In this scan the only part of the body exposed is the skin, which is less sensitive to radiation than other parts of the body. There is a very small risk of cancer from the x-rays in DEXA scan, but is too small to measure.

If you are pregnant you may not participate in this study. Unborn babies are more sensitive to radiation than children or adults.

As part of this study, you will be asked to sign a separate consent form to be in the part of this study involving genetic testing. Risks of genetic testing include the misuse of personal, genetic information. Although rare, misuse of such information has caused problems for persons related to

employment, life, or health insurance benefits and right. There is a risk that being in a genetics study can cause psychological distress or tension with other family members. Although there can be no absolute guarantees, every reasonable effort will be made to keep your personally identifiable information secret so that there will be no misuse. Even when the information is kept secret, if you are asked if you have ever been tested for a genetic disorder, answering "yes" could cause benefits to be denied or could cause other problems including discrimination.

If as part of this study you tell study staff that you plan to hurt yourself or someone else you should know what will happen. We will refer you for an evaluation by a mental health professional. You should also know the study doctor may have to report it to the authorities. There is a chance the authorities and the mental health professionals will find out that you are participating in this study.

For more information about risks and side effects, you should call the Principal Investigator, Michele K. Evans, M.D. at 410-558-8573.

ARE THERE ANY BENEFITS TO TAKING PART IN THE STUDY?

This study is not designed to provide direct benefits to any participants. If you agree to take part in this study, there may or may not be direct medical benefits to you. We hope the information learned from this study will benefit others in the future. There is no charge for any of the testing described. You may benefit by learning more about your health, or possibly from learning that you have a condition or problem. You will receive a Participant Report Package in the mail, with results of your visit to the MRVs. If the study doctor discovers any condition or problem, the information will be provided to you and your doctor, if you authorize it. To authorize the reporting of results to your physician you will need to sign a form called "Release of Medical Information". You will be asked to sign this form only if you want us to communicate with your physician. The study doctors do not provide medical treatment.

WHAT OTHER OPTIONS ARE THERE?

There are no other options associated with your participation in this study. You may choose either to participate or not to participate in this research. Taking part in this study is entirely voluntary. You may choose to withdraw from the study at any time.

WHAT ABOUT CONFIDENTIALITY?

Your personal health information (PHI) will be kept private to the degree allowed by law. You will not be identified by name in any publications resulting from this study. You will be asked to sign a separate form that will give permission to the investigator, the sponsor, and certain other people, agencies or groups to look at and review the records related to this study. The review could include

your personal health information. It could also include information discovered during this study. If you do not wish to sign this permission form you will not be allowed to participate in this study.

Personal Health Information (PHI) is stored in secure databases. These databases are password protected and maintained on a secure NIA/NIH system with access limited to authorized NIA staff. All NIA staff that has access to these databases has the proper training on patient confidentiality as well as the required Human Subject Protection Training. The system is administered using the security policies and regulations required by the National Institutes of Health consistent with the Health and Human Services Privacy Rule and HIPAA.
Organizations that may request inspect and/or copy your research and medical records for quality assurance and data analysis include groups such as: the National Institute on Aging, Office of Human Research Protection, MedStar Research Institute, Institutional Review Board (IRB).

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this certificate the researchers cannot be forced to disclose information that may identify you, even by court subpoena, in any federal, state, or local civil, criminal, administrative, legislative or other proceedings. The researchers will use the certificate to resist any demands for information that would identify you, except as explained below.
The Certificate cannot be used to resist a demand for information from personnel of the U.S. Department of Health and Human Services that is used for auditing or program evaluation or for information that must be disclosed in order to meet federal regulations.

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researcher may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from disclosing voluntarily, without your consent, information that would identify you as a participant in the research project under the following conditions: It does not apply to state requirements to report certain communicable diseases. In addition, the study doctor may be required to report certain cases of abuse, neglect, or suicidal or homicidal intent to the appropriate authorities.

WILL I BE PAID FOR PARTICIPATING IN THIS STUDY?

You will be paid $160 for participating in this phase (MRV visit) of the study. You will receive your payment in the form of an ATM debit card at the end of the MRV visit. If you are unable to complete all of the tests you may receive a portion of the payment. If you have to return to the MRVs to complete testing on another day, you could be compensated for the additional visit. The ATM card will be activated before you leave the vehicle. You will be able to take the card to an ATM machine

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in your neighborhood to withdraw your payment. We will provide round-trip transportation from your home to our mobile testing center if you want it. We will serve a box breakfast and box lunch if you are participating in tests during mid-day. We will do our best to meet your dietary needs if you have any.

WHAT ARE THE COSTS?

You do not have to pay anything to be in this study. However, if taking part in this study leads to procedures or care not included in the study, it may lead to added costs for you or your insurance company. You will not be charged for any tests or procedures that are part of this research study.

WHAT IF I'M INJURED OR BECOME ILL DURING THE STUDY?

We will make every effort to prevent injuries and illness from being in the study. If you have any adverse experience resulting directly from the study, the National Institute on Aging *will* provide or pay for short-term medical care for any injury resulting from participation in research here as long as the costs are not covered by your medical or hospital insurance. You should not expect any one to pay you for pain, worry, lost income, or non-medical care costs that occur from taking part in this research study. No other form of compensation is available for any adverse experience. *The National Institute on Aging, National Institutes of Health, the Federal Government, the MedStar Research Institute, MedStar Health,* do not have money set aside to repay you in case of injury.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

You have the right to be told about the nature and purpose of the study;
You have the right to be given an explanation of the exactly what will be done in the study and given a description of potential risks, discomforts, or benefits that can reasonably be expected;
You have the right to be informed of any appropriate alternatives to the study, including, if appropriate, any drugs or devices that might help you, along with their potential risks, discomforts and benefits;
You have the right to ask any questions you may have about the study;
You have the right to decide whether or not to be in the study without anyone misleading or deceiving you; and
You have the right to receive a copy of this consent form.

By signing this form, you will not give up any legal rights you may have as a research participant. You may choose not to take part in or leave the study at any time. If you choose to not take part in or to leave the study, you will not lose any of the benefits you would have received normally. We will tell you about new information that may affect your health, welfare, or willingness to be in this study.

WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, contact the investigator, Michele K. Evans, M.D., at (410)-558-8573. For medical assistance during the evening or on weekends, call the NIA Security Office at (410) 558-8119 and request that they contact the NIA Physician-on-Call.

If you are having a medical emergency, you should call 911 or go directly to the nearest emergency room.

If you are injured as a result of being in a study, or think you have not been treated fairly, please contact the NIA Clinical Director or Deputy Clinical Director at (410) 350-3922.

For questions about your rights as a research participant, you can call or write the following:

NIA Clinical Director
3001 S. Hanover Street, 5th Floor
Baltimore, MD 21225
Phone (410) 350-3922

NIA Clinical Research Protocol Office
3001 S. Hanover Street, Room 539
Baltimore, MD 21225
Phone: (410) 350-3947
Fax: (410) 350-3979.

MedStar Research Institute
Office of Research Integrity
6495 New Hampshire Avenue, Suite 201
Hyattsville, MD 20783
Phone: (301) 560-7339
Toll Free: (800) 793-7175
Fax: (301) 560-7336

SIGNATURES

As a representative of this study, I have explained the purpose, the procedures, the possible benefits and risks that are involved in this research study. Any questions that have been raised have been answered to the individual's satisfaction.

IRB number: 2009-149 **Clinical Site IC Version:** 05/27/2009
Project Title: **Healthy Aging in Neighborhoods of Diversity across the Life Span Wave 3 Phase 1**
Principal Investigator: MK Evans & AB Zonderman **Institution:** National Institute on Aging, NIH

Signature of Person Obtaining Consent _____ Date of Signature _____

I, the undersigned have been informed about this study's purpose, procedures, possible benefits and risks, and I have received a copy of this consent. I have been given the opportunity to ask questions before I sign, and I have been told that I can ask other questions at any time. I voluntarily agree to be in this study. I am free to stop being in the study at any time without need to justify my decision and if I stop being in the study I understand it will not in any way affect my future treatment or medical management. I agree to cooperate with Dr. Michele K. Evans, Dr. Alan B. Zonderman, .and the research staff and to tell them immediately if I experience any unexpected or unusual symptoms.

Participant's Signature _____ Date of Signature _____

Signature of Witness _____ Date of Signature _____

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A.3 HANDLS University of Delaware IRB Administrative Review

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE: January 23, 2017

TO: Marie Kuczmarski, PhD
FROM: University of Delaware IRB (HUMANS)

STUDY TITLE: [129457-11] Helthy Aging in Neighborhoods of Diversity Across the Life Span

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: January 23, 2017
EXPIRATION DATE: November 30, 2017
REVIEW TYPE: Administrative Review

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Administrative Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

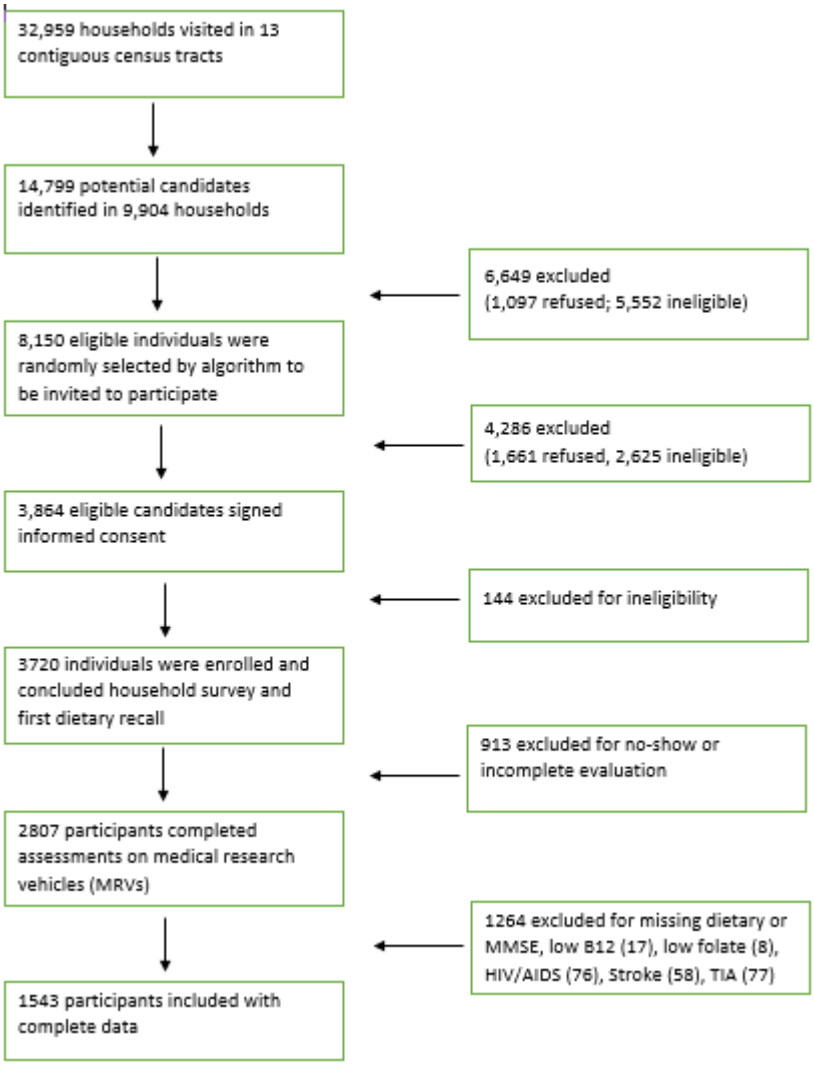
Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.

Appendix B

CONSORT CHART



Appendix C

DATA TABLES

C.1 Final Sample vs Not in Final Sample Basic Characteristics

	Total sample n= 2807		Not in Final sample n=1264		Final Sample n=1543		P value between Final and Not Final sample
	Weighted proportion or mean (SE)	95% CI	Weighted proportion or mean (SE)	95% CI	Weighted proportion or mean (SE)	95% CI	
Race (% white)	36.04 (0.0142)	31.90-40.27	35.98 (0.0214)	31.90-40.27	36.09 (0.0189)	32.47-39.88	0.9693
Sex (% females)	54.48 (0.0162)	51.28-57.64	55.14 (0.0248)	50.24-59.95	53.93 (0.0214)	49.71-58.09	0.7108
Poverty status (% >125%)	80.15 (0.0097)	78.18-81.98	80.49 (0.0144)	77.50-83.17	79.86 (0.0131)	77.18-82.31	0.7469
Mean age (years)	47.03 (0.2980)	46.45-47.62	47.51 (0.4952)	46.54-48.48	46.64 (0.3637)	45.93-47.35	0.1566

C.2 Confidence Intervals of Cognitive Test Scores for Total Sample and by Race

Cognitive tests	Total sample n= and 95% CI		Whites n= and 95% CI		African Americans n= and 95% CI	
	Wave 1	Wave 3	Wave 1	Wave 3	Wave 1	Wave 3
MMStot	n=1532	n=1025	n=673	n=429	n=859	n=596
	27.79-28.15	27.88-28.20	28.36-28.69	28.38-23.71	27.39-27.91	27.56-27.98
CVLtca	n=1299	n=955	n=558	n=405	n=741	n=550
	24.63-25.96	19.82-21.23	26.45-28.41	21.40-23.68	23.26-24.99	18.49-20.27
CVLfrl	n=1256	n=907	n=538	n=382	n=718	n=525
	7.27-7.90	5.72-6.36	8.13-8.98	6.63-7.73	6.64-7.48	5.03-5.79
BVRTot	n=1506	n=1010	n=661	n=423	n=845	n=587
	4.81-5.64	6.89-7.79	4.47-5.45	5.68-6.67	4.79-5.96	7.36-8.62
Attention	n=1310	n=946	n=562	n=408	n=748	n=538
	6.54-6.95	6.58-6.95	7.26-7.74	7.01-7.55	6.06-6.61	6.24-6.73
Fluency Word	n=1524	n=1028	n=663	n=429	n=861	n=599
	18.83-19.80	19.26-20.30	20.71-22.37	20.61-22.35	17.52-18.58	18.23-19.51
Digit span Fwd	n=1498	n=947	n=654	n=390	n=844	n=557
	7.25-7.61	7.35-7.80	7.74-8.27	7.91-8.61	6.89-7.35	6.94-7.49
Digit span Bck	n=1487	n=948	n=654	n=389	n=833	n=559
	5.65-6.01	5.76-6.15	6.51-7.02	6.43-7.09	5.07-5.52	5.32-5.76
Clock command	n=1529	n=1018	n=671	n=428	n=858	n=590
	8.70-8.92	8.76-8.98	8.94-9.20	8.87-9.14	8.51-8.82	8.65-8.95
TrailsAtest Sec	n=1475	n=1011	n=654	n=426	n=821	n=585
	32.85-35.68	32.14-37.70	27.67-29.98	28.22-31.77	35.36-39.52	33.41-41.85
TrailsBtest Sec	n=1474	n=952	n=654	n=405	n=820	n=547
	116.72-135.34	103.77-129.97	80.87-99.47	72.41-85.65	133.43-160.47	118.53-157.44

Abbreviations: MMStot, Mini-Mental State Exam total score; CVLtca, California Verbal Learning test total correct List A immediate recall; CVLfrl, California Verbal Learning test # correct List A long-delay free recall; BVRTot, Benton Visual Retention test total errors; Attention, Brief test of Attention total correct; Fluency Word, Animal Fluency total words; Digit span Fwd, Wechsler Adult Intelligence Scale-Revised Digit Span Forward total score; Digit span Bck, Wechsler Adult Intelligence Scale- Revised Digit Span Backward total score; Clock command total score; TrailsAtest Sec, Trails A test seconds; TrailsBtest Sec, Trails B test seconds. **Note: For BVRT and the Trails A and B lower numbers are better**