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Intracranial Atherosclerotic Stenosis and Its Association With Inflammation and Meditarrenean Diet

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INTRACRANIAL ATHEROSCLEROTIC STENOSIS AND ITS ASSOCIATION WITH INFLAMMATION AND MEDITARRENEAN DIET

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DEDICATION

To those who believed. Thank you to my family, mentors, committee, and friends who have guided me throughout this journey. I am grateful to you for all your support.

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ABSTRACT

Intracranial Atherosclerotic Stenosis (ICAS) is associated with 8 to 10% of all strokes in the U.S. Although there is some evidence that in the Asian population inflammation plays a role in asymptomatic ICAS, it has not been shown in the U.S. population. Prior studies have shown associations between diet and regulation of inflammation. Mediterranean dietary pattern has been associated with lower levels of inflammation and cardiovascular disease (CVD). In light of the important role of inflammation in intermediate stroke risk factors including atherosclerotic disease, and potential role of diet in modulating inflammation, understanding individuals' diets according to their inflammatory properties could yield important information about the links between diet, inflammation, and ischemic stroke. Our objectives for this study were two-fold. First, we assessed if high sensitivity C-reactive protein (hs-CRP), a marker of inflammation is associated with asymptomatic ICAS. Second, we assessed if Mediterranean dietary adherence is associated with ICAS, and if this association is modified with individual's inflammatory state.

Data came from the Atherosclerosis Risk in Communities Cohort, a community-based, prospective cohort total of 15,792 participants, aged 45 to 64 years recruited in1987 to 1989. We included 1,445 participants from this cohort who attended Visit 5 (2011-2013) and underwent high resolution MR angiography (MRA). MRA images were analyzed in a centralized lab to assess ICAS (outcome) and ICAS was graded as: no

stenosis, <50% stenosis, ≥50% stenosis/complete occlusion. Change in hs-CRP exposure) was categorized as sustained low/moderate (<3 mg/L at visits 2 and 4); decreased (≥3 mg/L at visit 2 and <3 mg/L at visit 4); increased (<3 mg/L at visit 2 and ≥3 mg/L at visit 4); and sustained elevated (≥3 mg/L at both visits). Multinomial logistic regression models were used to assess the association of 6-year change in hs-CRP with ICAS at visit 5. We also tested for the association between hs-CRP levels at each visit (categorized as <1, 1-3 and >3 mg/l) and ICAS. Further to assess Mediterranean dietary adherence we constructed an adapted Mediterranean Dietary Score (aMDS), that included the following 11 food components: non-refined cereals (whole grains), fruits, vegetables (excluding potatoes), nuts, legumes, fish, monounsaturated-to-saturated fat ratio (as an alternative for olive oil), red and processed meats, dairy products, poultry and alcohol. The aMDS ranged from 0 to 55, with higher values indicating greater adherence to the Mediterranean diet. Multinomial logistic regression models were used to assess the association between the Tertiles of the aMDS and ICAS.

Our findings strongly suggest participants with elevated hs-CRP over a period of 6 years had a strong association with both moderate and severe ICAS. Participants with increased hs-CRP over this 6 years period had increased odds for severe ICAS, however those with deceased hs-CRP did not. Further, when assessing association between Mediterranean diet and ICAS our results were somewhat suggestive of decreased burden of ICAS with Mediterranean diet adherence however, these findings were not statistically significant. Our findings also suggest inflammation (as measured by hs-CRP) may be an effect modifier for the association between Mediterranean diet and ICAS, with those with higher hs-CRP may benefit from Mediterranean diet adherence.

Elevated hs-CRP was positively associated and asymptomatic ICAS after controlling for potential confounders in a US population-based community study. Inflammation may be a risk factor for ICAS in this group, and in those with chronic inflammatory state adherence to Mediterranean diet can be beneficial.

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CHAPTER 1

INTRODUCTION

1.1 Statement of the problem

Stroke is the third leading cause of death in the United States. More than 140,000 people die each year from stroke in the United States (CDC). Recent data from the American Heart Association indicate that strokes account for about one of every 18 deaths in the United States, whereas on average someone has a stroke every 40 seconds. Despite recent advances in acute stroke treatment, effective primary stroke prevention, by means of improved control of vascular risk factors, has the greatest potential to reduce its burden (1).

Carotid stenosis and intracranial atherosclerotic disease (ICAD) are well-established vascular risk factors for ischemic stroke. Previous epidemiological studies have shown inflammation is closely linked with atherogenesis. High sensitivity C-reactive protein (hs-CRP), an indicator of inflammation, has been shown to be closely related to vascular disease burden, including carotid stenosis, and more recently with ICAD (2). A large prospective community-based study conducted in China, showed hs-CRP is an independent predictor of intracranial atherosclerotic burden (2). In this study, after adjusting for possible risk factors, high level of hs-CRP ($\geq$ 3 mg/l) remained significantly associated with asymptomatic ICAD (Adjusted Odds Ratio: 1.28, 95% CI: 1.02–1.61).

A mounting body of evidence from observational studies has shown associations between diet and regulation of inflammation (hs-CRP and other pro-inflammatory cytokines) (5-9). The western diet, rich in red meat, processed and artificially sweetened food products, refined grains, high fat-dairy products, and sugar-sweetened beverages has been linked with increased levels of inflammatory biomarkers and increased risk for CVD (10). On the contrary, a Mediterranean dietary pattern, rich in nuts, olive oil, legumes and fish, fruits and vegetables, moderate intake of wine, has been associated with lower levels of inflammation and a reduced risk of CVD (11-14).

In light of the important role of inflammation in intermediate stroke risk factors including atherosclerotic disease, and potential mediation of inflammation by diet, classifying individuals' diets according to their inflammatory properties could yield important information about the links between diet, inflammation, and ischemic stroke.

Given the anti-inflammatory properties of Mediterranean diet, at least in part the association between a Mediterranean diet and risk of stroke may be mediated by intracranial atherosclerotic disease (ICAD). However, to our knowledge the direct relationship between Mediterranean diet and ICAD has not been studied before.

1.2 Clinical significance

The relationship between diet and cardiovascular disease is well recognized, however relationship between dietary modulation of inflammation and its association with CVD is an emerging concept and has not been extensively studied, and not too much emphasis is given in clinical practice. More specifically, dietary modulation of inflammation, and its association with ICAD, an intermediate stroke risk factor, to our knowledge has never been previously studied. Classifying individuals' diets according to their inflammatory properties could yield important information about the links between

diet, inflammation, and cardiovascular disease burden. This knowledge can be useful in planning preventive strategies, by modulating and targeting dietary lifestyle changes, to reduce cardiovascular risk factors.

1.3 Study objective, specific aims, and hypothesis

Our objectives for this study were two-fold. First, we assessed if high sensitivity C-reactive protein, a marker of inflammation is associated with asymptomatic ICAS. Here, we analyzed if change in hs-CRP (exposure) over a period of 6 years was associated with ICAS. Second, we assessed if Mediterranean dietary adherence is associated with ICAS, and if this association is modified with individual's inflammatory state. We developed an adapted Mediterranean dietary score, to reflect an individual's adherence to Mediterranean dietary patter diet and developed adjusted regression model to assess its association with ICAS.

Our specific study aims for this project were:

Aim 1: To assess if hs-CRP, a marker for inflammation, is associated with late-life ICAD in a representative US population. **Hypothesis:** Inflammation is known to increase risk for atherosclerotic disease. We hypothesized inflammation would be associated with increased risk for intracranial atherosclerotic disease.

ii) Aim 2: Validation of modified Mediterranean Score in American Diet. **Hypothesis:** Modified Med Score would have robust positive and inverse associations with known dietary biomarkers validated in previous literature.

iii) Aim 3: To study the association between Mediterranean diet adherence and intracranial atherosclerotic stenosis and assess if this association modified by inflammation in a representative US population. **Hypothesis:** Mediterranean diet adherence is known to be associated with reduced inflammation, reduced oxidative stress,

improved lipid profile, improved blood pressure, and weight reduction. All these markers are associated with atherosclerotic disease. We hypothesized adherence to Mediterranean diet would be associated with a lower burden of ICAS, and individuals with higher inflammatory markers may be benefit more from Mediterranean diet adherence.

1.4 Study Outline

Chapter 2 of this dissertation provides details for our study design and analyses dedicated towards our first study Aim. Here we assessed our hypotheses that high sensitivity C-reactive protein is associated with asymptomatic ICAS in the U.S. population. Chapter 3 narrates our construction of an alternative Mediterranean dietary pattern score and its validation with known biomarkers. This would serve as an intermediate step for our next aim. Chapter 4 will be the manuscript for Aim 3 "study the association between Mediterranean diet adherence and intracranial atherosclerotic stenosis and assess if this association modified by inflammation in a representative US population." Chapter 5 includes a summary of each of the findings and an overall synthesis of the results, suggestions for future research, and conclusions.

CHAPTER 2

ASSOCIATION OF SUSTAINED INCREASED HIGH SENSITIVITY C-REACTIVE PROTEIN WITH INTRACRANIAL ATHEROSCLEROTIC STENOSIS IN THE US POPULATION

2.1 Abstract

Introduction and Hypothesis: Intracranial Atherosclerotic Stenosis (ICAS) is associated with 8 to 10% of all strokes in the U.S. Although there is evidence that in the Asian population inflammation plays a role in asymptomatic ICAS, it has not been shown in the U.S. population. We assessed the hypotheses that high sensitivity C-reactive protein (hs-CRP), a marker of inflammation is associated with asymptomatic ICAS.

Methods: The Atherosclerosis Risk in Communities Cohort is a community-based, prospective cohort total of 15,792 participants, aged 45 to 64 years recruited in1987 to 1989. We included 1,445 ARIC participants attended Visit 5 (2011-2013) and underwent high resolution MR angiography (MRA), and also had data for hs-CRP measured at visits 2 (1990-92) and 4 (1996-98). MRA images were analyzed in a centralized lab to assess ICAS (outcome) and ICAS was graded as: no stenosis, <50% stenosis, ≥50% stenosis/complete occlusion. Change in hs-CRP (exposure) was categorized as sustained low/moderate (<3 mg/L at visits 2 and 4); decreased (≥3 mg/L at visit 2 and <3 mg/L at visit 4); increased (<3 mg/L at visit 2 and ≥3 mg/L at visit 4); and sustained elevated (≥3 mg/L at both visits). Multinomial logistic regression models were

used to assess the association of 6-year change in hs-CRP with ICAS at visit 5. We also tested for the association between hs-CRP levels at each visit (categorized as <3 and ≥3 mg/l) and ICAS.

Results: Mean age of study participants (at visit 4) was 63±6, 42% were male, 81% were Caucasian and 19% were African-American. Compared to the participants with sustained low/moderate hs-CRP over the 6 years period, those with sustained elevated hs-CRP were more likely to have both moderate and severe ICAS (ORs [95% CIs]: 1.57 [1.10, 2.27] and 1.66 [1.03, 2.84], respectively). Persons with increased hs-CRP (<3 to ≥3 mg/L) had increased odds for severe ICAS (but not moderate ICAS), however those with decreased hs-CRP did not. Associations for elevated hs-CRP level (>3 mg/l at any visit) and ICAS were greater for those with increased hs-CRP at Visit 4, but not at Visit 2.

Conclusions: Elevated hs-CRP was positively associated and asymptomatic ICAS after controlling for potential confounders in a US population-based community study. Inflammation may be a risk factor for ICAS in this group.

2.2 Introduction

Intracranial Atherosclerotic Stenosis (ICAS) is associated with 8 to 10% of all strokes in the United States (U.S.) (15,16) and 30%-50% of strokes in Asian population (17), making it one of the most common risk factors for stroke worldwide (18). Among patients with severe ICAS, the annual risk of recurrent stroke is ~14% despite targeted medical management (19).

Several epidemiological studies have shown geographic and ethnic differences in the prevalence of ICAS. ICAS is more frequently encountered in Asians, whereas extracranial carotid artery disease is more common in Western populations (20,21). Data

from epidemiological studies from the U.S. suggest ICAS preferentially affects Hispanics and Blacks as compared to Whites (15,16). Etiology of such ethnic differences is not clear, however environmental, and genetic factors have been implicated.

Inflammation, as measured by proinflammatory biomarkers like high sensitivity C-reactive protein (hs-CRP), has been shown to be closely related to cardiovascular events such as acute coronary syndrome, coronary artery disease, peripheral vascular disease, metabolic syndrome and stroke (21-26). Data for the role of inflammation as a risk factor for the development and progression for ICAS is limited, especially in the Western population. More recently, a community-based study conducted with an Asian population, showed hs-CRP is an independent predictor of asymptomatic ICAS (27). Although there is evidence in the Asian population inflammation plays a role in asymptomatic ICAS, to our knowledge this has not been studied in the U.S. population.

Considering possible role of the environmental and genetic factors that influence the geographical and racial disparities on the burden of ICAS, a better understanding of such associations in the U.S population would strengthen of scientific evidence, and help identify populations at risk that can potentially be targeted for medical and lifestyle interventions. We assessed the hypotheses that high sensitivity C-reactive protein (hs-CRP), a marker of inflammation is associated with asymptomatic ICAS in the U.S. population.

2.3 Methods

Study Population

The ARIC study is a community-based, prospective cohort of 15,792 black and white participants, aged 45 to 64 years recruited in 1987 to 1989 randomly selected from

4 communities (Forsyth County, NC; Jackson, MS; suburbs of Minneapolis, MN; and Washington County, MD). First visit (baseline) took place from 1987 to 1989, with 4 subsequent visits: Visit 2 (1990–1992); Visit 3 (1993–1995); Visit 4 (1996–1998), and Visit 5 (2011–2013). Among 10,036 participants who were still alive at the time of study, 6538 (65%) took part in Visit 5. High-resolution magnetic resonance angiography (MRA) was offered to an age-stratified random sample of the participants from Visit 5 (n = 1,959). Of these 1,704 participants completed the study per study protocol with adequate image quality. For our analysis, we excluded participants with missing information on hs-CRP (n = 235). We also excluded those with history of stroke (n=11) and nonwhite or nonblack participants (n=3), because of the small number. After excluding these, a total of 1,455 participants met our inclusion criteria and their data were included in our analysis.

Assessment and classification of Intracranial Atherosclerosis

A sub cohort of ARIC participants who attended visit 5, completed high resolution MRA per study protocol. Details of MRA protocols, quality control, and reliability have been reported elsewhere (28). Briefly, all scans were performed on high resolution 3-Tesla Siemens MRI scanners. Images were analyzed by seven certified readers who were blinded on the information on participant characteristics. Percent inter and intra-reader agreement for ordinal intracranial stenosis were 94.4% and 93.8%, respectively. Intracranial vascular territories were categorized as intracranial internal carotid artery (cavernous and supraclinoid segments), middle cerebral artery (M1-M3 segments), anterior cerebral artery (A1-A3 segments), posterior cerebral artery (P1-P3 segments), basilar artery, and vertebral artery (V4 segment), for right and left hemisphere

respectively. For each of the above intracranial vascular territories, the degree of stenosis was measured according to the criteria established in the Warfarin-Aspirin Symptomatic Intracranial Disease trial (i.e. no detectable stenosis, less than 50%, 51% to 70%, 71% to 99%, and complete occlusion) (29). For the purpose of our analysis these categories were consolidated into 3 groups: no stenosis, < 50% stenosis, $\geq$ 50% stenosis/complete occlusion.

Assessment and Classification of hs-CRP

For all study participants, who attended visit 2 and visit 4 blood sample was drawn after an 8 hour fasting period. Plasma levels of hs-CRP were determined by an immunoturbidimetric assay at visit 2 (Roche Diagnostics, Indianapolis, IN), and using a nephelometric method (Siemens Healthcare Diagnostics, Deerfield, IL). According to the guidelines from the American Heart Association, hs-CRP levels were categorized into three groups: low (hs-CRP <1 mg/l), moderate (hs-CRP 1–3 mg/l) and elevated (hs-CRP >3 mg/l) (30). To assess longitudinal changes in hs-CRP levels over a period of 6 years, we used the categories proposed by Parrinello et al. (31) : sustained low/moderate (hs-CRP <3 mg/L at both visits 2 and 4); decreased (≥3 mg/L at visit 2 and <3 mg/L at visit 4); increased (<3 mg/L at visit 2 and ≥3 mg/L at visit 4); and sustained elevated (≥3 mg/L at both visits 2 and 4). Additionally, we also assessed mean of the hs-CRP across Visit 2 and 4, and categorized this into Tertiles.

Covariates of interest

Date of birth, gender, and race were reported by study participants at baseline. Cardiovascular risk factors measured at visit 4 included alcohol use (current, past, or never), smoking history (current, past, or never), prevalent hypertension, prevalent

diabetes, prevalent cardiovascular disease (CVD), cholesterol-lowering medication use, BMI categories (normal, overweight and obese). We defined hypertension as blood pressure of 140/90 mm Hg or higher, or use of antihypertensive medications; diabetes as fasting glucose level of ≥ 126 mg/dL or non-fasting glucose level ≥ 200 mg/dL, or use of antidiabetic medications; and dyslipidemia as total cholesterol level ≥ 240 mg/dL, low-density lipoprotein cholesterol level ≥ 160 mg/dL, high-density lipoprotein cholesterol level ≤ 40 mg/dL, or triglyceride level ≥ 200 mg/dL, or use of cholesterol-lowering medications. Physical activity measures were assessed at visit 3 and these were used to calculate leisure time physical activity levels (LTPA). We categorized LTPA into 3 groups: (1) no LTPA; (2) less than minimum goal (<450 metabolic equivalent (MET) min/wk); (3) meeting or exceeding the minimum goal (greater than 450 MET min/wk).

Statistical analysis

We used SAS version 9.4 (SAS Institute Inc., Cary, NC) to conduct all statistical analyses. We compared baseline characteristics between the CRP groups, using the chi-square test for categorical variables and variance (ANOVA) for continuous variables. Multinomial logistic regression models were used to calculate odd ratios (ORs) of the effects of variables on the relationship between hs-CRP levels and ICAS. The variables adjusted for were age, gender, race, education level, leisure time physical activity, prevalent hypertension, cholesterol-lowering medication, body mass index, prevalent diabetes. All statistical analyses were two-tailed, and a P value of 0.05 was considered statistically significant.

2.4 Results

ICAS was prevalent in 30% of study participants and 10% had ICAS ≥50% (data not presented in the tables). The mean age of study cohort was 63 years at visit 4. Over a

6 years period (between visit 2 and 4), nearly 50% of the study population had sustained low or moderate hs-CRP (<3 mg/L at both visits) and about 25% of participants had sustained elevated hs-CRP (>3 mg/L at both visits) (Table 2.1). In comparison to those with sustained low or moderate hs-CRP, participants with sustained elevated hs-CRP were more likely to be female or black (38% vs. 22% and 76% vs. 48%, respectively) (Table 2.1). Participants with sustained elevated hs-CRP were more likely to have prevalent hypertension, diabetes, CVD and obesity, and were less likely to be physically active (Table 2.1). Participants who had a decrease in hs-CRP (>3 to <3 mg/L) over this period were more likely to be taking cholesterol-lowering medications at visit 4 (Table 2.1).

Participants with sustained elevated hs-CRP had increased odds for moderate and severe ICAS compared to those with sustained low/moderate hs-CRP (adjusted OR 1.57 and 1.66, respectively; Table 2.2). Increased hs-CRP over the course of 6 years was associated with increased odds for severe ICAS and moderate ICAS (adjusted OR 1.89 and 1.35), though the association for moderate ICAS was not statistically significant (Table 2.2).

Table 2.3 presents this analysis stratified by gender. There were no gender specific differences in odds for ICAS, except decreased in hs-CRP appeared to be protective for moderate ICAS in female (OR 0.44 vs. 1.48, for male and female respectively), though this was not statistically significant.

We performed a sensitivity analyses where we assessed the mean hs-CRP between Visit 2 and 4 and assessed its association with ICAS at Visit 5. For this analyses hs-CRP was categorized into Tertiles. Tertile 1 was categorized as mean hs-CRP level

less than 1.35 mg/dl, Tertile 2 as mean hs-CRP between 1.36 mg/dl and 3.58 mg/dl, and Tertile 3 as mean hs-CRP at or above 3.56 mg/dl. Results for the odd ratio for moderate and severe ICAS across Tertiles of mean hs-CRP are presented in Table 2.4. Briefly, participants in Tertile 2 and 3 had higher odds for moderate ICAS (aORs 1.52 [95 CI 1.07 – 2.16] and 1.53 [95 CI 1.06 – 2.21]) and those in Tertile 3 had higher odds for severe ICAS (aOR 1.97 (95 CI 1.16 – 3.34)

Participants with elevated hs-CRP had greater odds for severe ICAS compared to those with low hs-CRP (< 1 mg/l), though these estimates didn't meet statistical significance (adjusted OR 1.61 and 1.72, respectively) (Table 3). Elevated hs-CRP at visit 4, both in moderate (1-3 mg/l) and high (>3 mg/l) categories, was associated with increased odds for moderate ICAS (adjusted OR 1.45 and 1.99, respectively), however this was not case for elevated hs-CRP at visit 2 (Table 2.5).

2.5 Discussion

In this US population-based community study, we report a significant and independent associations between elevated hs-CRP and asymptomatic ICAS as assessed by high resolution MR Angiography. Our results show that sustained elevated-CRP (≥3 mg/L threshold) over a period of six years is a strong predictor for ICAS. Single measures of elevated hs-CRP at two different time points in the study were associated with increased odds for ICAS as well, however the most proximally measured value of hs-CRP with reference to ICAS ascertainment may be more important than past measurements. These findings support prior evidence that chronically high levels of inflammation play an active role in endothelial insult and in long-term development of atherosclerosis.

Prior studies have shown an association between hs-CRP and extracranial atherosclerosis (carotid and coronary artery) (21-26)), but data on association between inflammation and ICAS is limited, particularly in Western populations. In a Chinese cohort, Wang et. al. (27) showed elevated hs-CRP is a risk factor for ICAS. To our knowledge ours is the first study to find such an association in the US population. In comparison to the study design of Wang et. al., our study has several unique strengths. First, the use of high resolution MRA is a very sensitive and specific diagnostic method to assess ICAS (32,33). In comparison, Wang et. al used transcranial doppler (TCD) to measure the presence of ICAS. Although TCD is a convenient non-invasive screening method, it is not as accurate as an angiogram for determining ICAS (20). Furthermore, use of MRA not only improves the diagnostic accuracy, it also enabled us to measure the severity of ICAS. Second, we used two measures of hs-CRP to assess for "inflammation" among study participants over a period of time. In contrast, Wang et al. used a single measure of hs-CRP for their analyses. A single hs-CRP reading cannot account for its for its inherent short-term variability (35,36). Per American Heart Association recommendations, to reduce within-person variability while using hs-CRP as a marker for inflammation, at least two measurements should be used (37). Indeed, our analyses confirms as compared to a single measure, elevated sustained increases in hs-CRP had a more robust association with ICAS.

Despite being a large community-based cohort study with good sample size, our study has a few limitations. Firstly, we did not have baseline information for our study participants on the outcome of interest (ICAS), our results can only be interpreted as an association not temporal risk. Another potential limitation of this study is that time of

ICAS ascertainment and hs-CRP measurement were not concurrent, thus it is possible we may not have identified participants with significant changes in hs-CRP level from the time it was measured to when ICAS was ascertained. This limitation however was partially overcome by assessing CRP trends. Also, individuals who died over the 6-year period were not accounted for. As elevated CRP is associated with excess mortality and ICAS, this may have underestimated the associations reported in this study. Additionally, extracranial carotid stenosis was not assessed in this study, thus we cannot comment if the relationship between intra vs. extra-cranial stenosis is different. Despite these limitations, to our knowledge this is the first study to assess the relationship between ICAS and inflammation in the US population.

In conclusion, we found elevated hs-CRP to be an important predictor of ICAS in the US population. Our results suggest that chronically sustained high level of inflammation, as assessed through repeated measurements of hs-CRP is a stronger indicator of disease development. Therefore, among population at risk, such as those suffering with ischemic stroke, it is important to screen for ICAS for those with elevated hs-CRP levels. On the same note, while considering risk factor stratification for secondary stroke prevention in populations with known ICAS, it is also important to monitor hs-CRP levels, and pursue targeted treatments to address chronic inflammatory states. Further studies are needed to understand the mechanisms of hs-CRP in asymptomatic ICAS.

Table 2.1 Selected characteristics of the study population by six years change in hs-CRP measured at visit 2 (1990-92) or visit 4 (1996-98)

	Sustained Low/Moderate (<3 mg/L at both visits) (N=773, 53.5%)	Decreased (>3 to <3 mg/L) (N=108, 7.5%)	Increased (<3 to >3 mg/L) (N=201, 13.9%)	Sustained Elevated (>3 mg/L at both visits) (N=363, 25.1%)
	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %
Age	62.6 (5.7)	63.6 (5.9)	62.6 (5.6)	62.7 (5.6)
Female (%)	48.5 %	51.9 %	70.7 %	76.3 %
Race – Black (%)	22.1 %	25 %	24.4 %	38 %
Education				
<High School	11.64 %	9.26 %	13.93 %	15.15 %
High School or College	41.04 %	37.96 %	41.79 %	39.67 %
>College	47.09 %	52.78 %	44.28 %	45.18 %
Body Mass Index†	25.7 (3.7)	27.5 (4.2)	26.2 (4.3)	29.5 (5.6)
Hypertension (%)	25.5 %	34.3 %	24.8 %	46.4 %
Diabetes (%)	3.8 %	6.2 %	2.8 %	7.7 %
Dyslipidemia	32 %	36.5 %	27.8 %	35.1 %
Prevalent CVD	6.2%	11.5%	7.1%	11.8%
Smoking (%)				
Never	44.5 %	47.6 %	36.8 %	41.1 %
Former	46.7 %	42.9 %	49.8 %	48.7 %
Current	8.8 %	9.5 %	13.4 %	10.2
Alcohol (%)				
Never	52.1 %	51.9 %	54.1 %	36.2 %
Former	25.7 %	25.5 %	24.5 %	36 %
Current	22.1 %	22.6 %	21.4 %	27.8 %
Cholesterol Lowering Medications	13.7 %	21.3 %	8.0 %	9.1 %

Table 2.2 Association of six years change in hs-CRP measured at visit 2 (1990-92) or visit 4 (1996-98) with prevalent ICAS measured at visit 5 (2011-2013)

	Moderate ICAS			Severe ICAS		
	Cases/ Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model**)	Cases / Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model**)
Sustained Elevated (>3 mg/L at both visits)	89/363 (24.5%)	1.72 (1.22 - 2.41)	1.57 (1.10 - 2.27)	42/363 (11.6%)	2.14 (1.30 - 3.50)	1.66 (1.03 – 2.84)
Increased (<3 to >3 mg/L)	44/201 (21.9%)	1.37 (0.90 - 2.08)	1.35 (0.89 - 2.08)	26/201 (12.9%)	1.76 (0.98 - 3.17)	1.89 (1.07 - 3.24)
Decreased (>3 to <3 mg/L)	22/108 (20.4%)	0.91 (0.49 - 1.54)	0.84 (0.47 - 1.50)	9/108 (8.3%)	1.07 (0.51 - 2.52)	1.03 (0.48 - 2.45)
Sustained Low/Moderate (<3 mg/L at both visits)	146/773 (18.9%)	Reference	Reference	62/773 (8%)	Reference	Reference
Area under curve		0.56	0.61		0.66	0.69

*Crude logistic regression models adjusted for age and sex only.
** Logistic regression models were adjusted for the following covariates: age, gender, race, education level, leisure time physical activity (LTPA), prevalent hypertension, cholesterol-lowering medication, body mass index, prevalent diabetes.

Table 2.3 Association of six years change in hs-CRP measured at visit 2 (1990-92) or visit 4 (1996-98) with prevalent ICAS measured at visit 5 (2011-2013) by gender

		Moderate ICAS			Severe ICAS		
		Cases/ Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model**)	Cases / Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model**)
Sustained Elevated (>3 mg/L at both visits)	M	26/77 (33.8%)	2.01 (1.15 – 3.51)	1.91 (1.06 – 3.42)	12/77 (15.6%)	2.99 (1.36 - 6.55)	2.18 (0.93 -5.08)
	F	56/249 (22.5%)	1.68 (1.10 – 2.58)	1.48 (0.86 – 2.50)	23/249 (9.2%)	1.62 (0.87 - 3.01)	1.33 (0.65 - 2.71)
Increased (<3 to >3 mg/L)	M	14/53 (26.4%)	1.30 (0.66 – 2.58)	1.24 (0.60 - 2.52)	8/53 (15.1 %)	1.73 (0.68 - 4.38)	1.64 (0.61 -4.40)
	F	26/129 (20.2 %)	1.48 (0.87 – 2.52)	1.26 (0.86 – 2.50)	13/129 (10.1%)	1.70 (0.82 - 3.53)	1.73 (0.82 - 3.64)
Decreased (>3 to <3 mg/L)	M	6/48 (12.5%)	0.47 (0.19 – 1.17)	0.44 (0.17 – 1.09)	5/48 (10.4%)	1.01 (0.35 - 2.85)	0.86 (0.29 - 2.54)
	F	11/49 (22.5%)	1.56 (0.73 – 3.29)	1.48 (0.69 – 3.19)	4/49 (8.2%)	1.15 (0.37 - 3.58)	1.07 (0.32 -3.51)
Sustained Low/Moderate (<3 mg/L at both visits)	M	85/367 (8.2%)	Reference	Reference	30/367 (8.17%)	Reference	Reference
	F	52/339 (6.8%)			23/339 (6.8%)		

M: Male; F: Female

*Crude logistic regression models adjusted for age and sex only.

** Logistic regression models were adjusted for the following covariates: age, gender, race, education level, leisure time physical activity (LTPA), prevalent hypertension, cholesterol-lowering medication, body mass index, prevalent diabetes.

Table 2.4 Association of Tertiles of mean hs-CRP measured at visit 2 (1990-92) or visit 4 (1996-98) with prevalent ICAS measured at visit 5 (2011-2013)

	Moderate ICAS			Severe ICAS		
	Cases/ Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model*)	Cases / Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model**)
Tertile 1[a]	84/487 (17.3%)	Reference	Reference	37/487 (7.6%)	Reference	Reference
Tertile 2[a]	110/477 (23.1%)	1.50 (1.06 – 2.12)	1.52 (1.07 – 2.16)	41/477 (8.6%)	1.12 (0.66 – 1.90)	1.14 (0.66 – 1.95)
Tertile 3[a]	107/481 (22.3%)	1.65 (1.15 – 2.36)	1.53 (1.06 – 2.21)	61/481 (12.7%)	2.28 (1.38 – 3.79)	1.97 (1.16 – 3.34)
***AUC		0.71	0.74		0.61	0.64

[a]Tertile 1 was categorized as mean hs-CRP level less than 1.35 mg/dl, Tertile 2 as mean hs-CRP between 1.36 mg/dl and 3.58 mg/dl, and Tertile 3 as mean hs-CRP at or above 3.56 mg/dl.
*Crude logistic regression models adjusted for age and sex only.
** Logistic regression models were adjusted for the following covariates: age, gender, race, education level, leisure time physical activity (LTPA), prevalent hypertension, cholesterol-lowering medication, body mass index, prevalent diabetes.
***Area Under Curve

Table 2.5 Association of hs-CRP measured at visit 2 (1990-92) or visit 4 (1996-98) with ICAS assessed at visit 5 (2011-2013)

	Moderate ICAS			Severe ICAS		
	Cases/ Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model**)	Cases / Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model**)
Hs-CRP Visit 2						
< 1 mg/l	75/389 (19.3%)	Reference	Reference	28/389 (7.2%)	Reference	Reference
1 - 3 mg/l	115/587 (19.6%)	1.06 (0.75 - 1.49)	1.04 (0.73 - 1.48)	60/587 (10.2%)	1.47 (0.87 - 2.48)	1.44 (0.81 - 2.44)
> 3 mg/l	111/469 (23.7%)	1.43 (1.01 - 2.05)	1.29 (0.90 - 1.91)	51/469 (10.9%)	1.88 (1.09 - 3.25)	1.61 (0.89 - 2.92)
***AUC		0.57	0.61		0.65	0.68
Hs-CRP Visit 4						
< 1 mg/l	59/365 (16.1%)	Reference	Reference	31/365 (8.5%)	Reference	Reference
1 - 3 mg/l	109/516 (21.1%)	1.48 (1.01 - 2.22)	1.45 (0.99 - 2.15)	40/516 (7.8%)	0.94 (0.51 - 1.61)	0.98 (0.56 - 1.71)
> 3 mg/l	133/564 (23.6%)	2.06 (1.14 - 3.02)	1.99 (1.32 - 3.01)	68/564 (12.1%)	1.82 (1.09 - 3.06)	1.72 (0.99 - 3.05)
***AUC		0.61	0.63		0.68	0.73

*Crude logistic regression models adjusted for age and sex only.
** Logistic regression models were adjusted for the following covariates: age, gender, race, education level, leisure time physical activity (LTPA), prevalent hypertension, cholesterol-lowering medication, body mass index, prevalent diabetes. All covariates were visit 4 values, except for LTPA which was assessed at visit 3.
***Area under the curve

CHAPTER 3

DEVELOPMENT OF AN ADAPTED MEDITERRANEAN DIET SCORE AND ITS VALIDATION ON AN AMERICAN DIET

3.1 Introduction

Cardiovascular diseases are the leading cause of morbidity and mortality in the United States and worldwide. The Mediterranean diet is one of the best studied diets known for its cardiovascular health benefits and has remained a focus of research interest worldwide. Prior studies have shown adherence to a Mediterranean dietary pattern reduces risk of cardiovascular diseases, metabolic syndrome, type 2 diabetes, certain cancers, cognitive impairment, and dementia (38-43).

The traditional Mediterranean diet is characterized by a high intake of fish, whole grains, unsaturated fats, plant proteins, nuts, legumes, fruits and vegetables, moderate intake of alcohol, and low consumption of red meat, refined grains, and sweets (44). The Mediterranean-style dietary recommendations as reflected by the Mediterranean diet pyramid, are based on the traditional dietary pattern from the Greek island of Crete (44,45).

The Mediterranean diet has been found to be an alternative to a healthy dietary pattern in the US population (46). Indexes developed by Trichopoulou et al (42) and Panagiotakos et al. (47) to quantify adherence to Mediterranean diet are well known.

These scales were developed and studied on the population residing in the Mediterranearegion. In the non- Mediterranean populations, the food items consumed may vary a lot from a traditional Mediterranean dietary pattern, making it a challenge to accurately assess an individual's diet using the same instruments. Thus a few modified and improvised indexes have been proposed in nutritional epidemiologic studies to assess adherence to Mediterranean diet based on the study population characteristics and dietary habits (48-51).

In this study, we developed an adapted Mediterranean diet score (aMDS) to assess Mediterranean diet adherence and its associations in among participants of the Atherosclerosis Risk in Communities Cohort. The purpose of this research is to describe the methodology in development of the aMDS and to examine the content validity of this score for an American Diet among participants of the Atherosclerosis Risk in Communities Cohort.

3.2 Methods

Study population

The ARIC study is a community-based prospective cohort of predominantly biracial (black and white) participants aged 45 to 64 years recruited in 1987 to 1989 randomly selected from 4 communities (Forsyth County, NC; Jackson, MS; suburbs of Minneapolis, MN; and Washington County, MD). Dietary intake was assessed by an interviewer-administered 66-item semi-quantitative food frequency questionnaire at baseline visit (1987–1989). Our analytical sample consisted of 12,404 persons from the baseline visit who completed the food frequency questionnaire, after excluding those (n=404) with the missing nutritional data.

aMDS Components

The aMDS was adapted from the Mediterranean diet scores proposed by Trichopoulou et al (42) and Panagiotakos et al. (47). For the calculation of aMDS we included the following 11 food components: non-refined cereals (whole grains), fruits, vegetables (excluding potatoes), nuts, legumes, fish, monounsaturated-to-saturated fat ratio (as an alternative for olive oil), red and processed meats, dairy products, poultry and alcohol.

We assigned scores in the range from 0 to 5 for the weekly consumption of each of these food components. Each component of the aMDS was calculated based on the updated recommended intakes of food in the Mediterranean diet pyramid (52).

Items assumed to be contributing towards recommended Mediterranean dietary pattern received an incremental score ranging from 0 to 5, whereas consumption of food items against the Mediterranean dietary pattern received scores on the inverse ordinal scale (Table 1). For example, scores assigned to vegetable consumption (times/week) ranged from 0 to 5 for the following categories: never, 1 to 6 times, 7 to 12 times, 13 to 20 times, 21 to 32 times and $\geq$ 6 times. However in contrast to the indexes proposed by Trichopoulou et al and Panagiotakos et al, we excluded potatoes from vegetables because of differences in preparation methods in the United States and in Europe (53).

Nut consumption was not included by the scale proposed by Panagiotakos et al, and nuts were included as a combined category with fruits in the scale proposed by Trichopoulou et al. We included nuts as a separate group in our index, and scores in the range from 0 to 5 were assigned for the recommended weekly intakes. For alcohol consumption, gender specific cut-offs were applied: a score of 5 was assigned for

consumption of less than 28 g and 14 g of alcohol per day, score 0 for no consumption or for consumption of greater than 70 g and 28 g per day in men and women, respectively, and the cutoffs for subcategories between 0 and 5 were reassigned with even intervals (54).

Olive oil consumption, an important component of Mediterranean diet, was not measured in the FFQ used in ARIC. In our score we used the ratio of MUFAs to SFAs as a representation of the olive oil component. The ratio was then divided into 6 even categories based on percentiles, and a score of 0 to 5 was assigned in an incremental scale. The aMDS ranged from 0 to 55, with higher values indicating greater adherence to the Mediterranean diet (Table 1).

Content Validity of thre aMDS

We assessed content validity of the aMDS, based on certain nutrients that have been documented in literature to be different in Mediterranean diet than Western diet. For instance, nutrients such as n-3 fatty acids (ω-3), unsaturated fats, dietary fibers, certain vitamins are rich in Mediterranean diet. We hypothesized nutrient contents of a traditional Mediterranean diet will correlated with aMDS. List of all variables that were tested included total daily intake of energy, fiber, saturated fat, trans fat, monounsaturated fat, oleic acid (18:1), polyunsaturated fat, n-6 fatty acids, n-3 fatty acids, ratio (n-6):(n-3) fatty acids, linoleic acid (LA) (18:2), α-linolenic acid (ALA) (18:3), alcohol, eicosapentaenoic acid (EPA) (20:5), docosahexaenoic acid (DHA) (22:6), ß- Carotene, lycopene, folate, Vitamins B6, C, D, E, calcium, magnesium, potassium, and high sensitivity C-reactive protein (hs-CRP). Higher quintiles of aMDS were inversely

associated with saturated fat, trans fat, oleic acid, the ratio (n-6):(n-3) fatty acids, and hs-CRP.

Statistical Methods

We compared baseline characteristics between the aMDS quintiles, using the chi square test for categorical variables and variance (ANOVA) for continuous variables. To assess p-value for trends for the nutrients across aMDS quintiles, a simple linear regression test was used.

3.3 Results

General and clinical participant characteristics of ARIC population according to quintiles of the aMDS are shown in Table 2.2. Higher quintiles of MDS were characterized by larger proportions of individuals with high educational levels. Individuals in the higher MDS quintiles were less likely to be active smokers, had a lower mean BMI, and had lower prevalence of existing chronic conditions including hypertension, diabetes mellitus and stroke (Table 2.2).

The mean aMDS score for the ARIC cohort at the baseline visit was 26.3 (SD ± 5.3), out of maximum possible score of 55. Among the 11 food groups considered in the aMDS, median component scores were highest for poultry, dairy, fruits, and the ratio for MUFAs to SFAs (Table 2.3). The proportion of population that attained the maximum score of 5 for an individual food component, was highest for poultry (55%), followed by dairy (25%), alcohol intake (24% and 18% for men and women, respectively), and the ratio for MUFAs to SFAs (18%). The majority of ARIC population failed to meet the recommended intake of a food group (Table 2.3).

The associations between aMDS quintiles and selected nutrients previously shown in literature to corelate to the Mediterranean diet are shown in Table 3. Higher quintiles of aMDS were found to have a significant and positive association with dietary fiber, polyunsaturated fat, n-3 fatty acids (linolenic acid (18:3), eicosapentaenoic acid (20:5), and docosahexaenoic acid (22:6)), alcohol, beta carotene, lycopene, folate, Vitamins B6, C, D, E, magnesium and potassium. Whereas, higher quintiles of aMDS were inversely associated with saturated fat, trans fat, oleic acid, the ratio (n-6):(n-3) fatty acids, hs-CRP. In contrast to these expected associations consistent with previous studies, we observed an unexpected positive association between the aMDS and total energy intakes and n-6 fatty acids, and an unexpected inverse association with monounsaturated fat and calcium intake.

3.4 Discussion

The aMDS index based on frequently eaten food that was adapted for the ARIC population reflected nutrients related to the Mediterranean diet. For example, individuals in the higher quintiles of the aMDS index had higher mean intakes of nutrient that were beneficial for health such as dietary fiber, polyunsaturated fat (relative), n-3 fatty acids, beta carotene, lycopene, folate, Vitamins B6, C, D, E. Whereas, higher quintiles of aMDS were inversely associated with saturated fat, trans fat, oleic acid, the ratio (n-6):(n-3) fatty acids, hs-CRP.

Several indexes to assess Mediterranean diet adherence have been previously reported. The Mediterranean diet scores developed by Trichopoulou et al. (42) and Panagiotakos et al. (47), are widely known. These indexes included 9 and 10 food components respectively, and were developed and validated based on the natives Mediterranean population. Few adapted scores have been since proposed on non-

Mediterranean populations, such as e S-MDS (55), MedDQI (47), the alternate MD score (50), and Japanese-adapted MD score (51). The components of these indexes and their scoring systems differ across studies.

In this study we developed a simple Mediterranean diet score adapted to an American diet in the Atherosclerosis Risk in Communities Cohort based on the inherent characteristics of the Mediterranean diet. The score was developed based on the recommended intake of food in the updated Mediterranean diet pyramid (52). We excluded potatoes from vegetables because the way potatoes are prepared in U.S. is quite different from Mediterranean countries (53). We did not incorporate olive oil in our score, because the FFQ used in the ARIC study did not capture this item. In addition, consumption of olive oil is low in the United States. As an alternate, we used the ratio of monounsaturated fatty acids to saturated fatty acids as an indirect parameter, which has been used in prior studies (42, 50, 56, 57).

To test of content validity, this score was compared against various nutrients and markers previously shown to be closely related to the Mediterranean diet. All the selected nutrients had the expected positive or inverse associations with our instrument, except for the total energy intakes and n-6 fatty acids and monounsaturated fat. Monounsaturated fat is present in meat and may reflect higher meat consumption in the American diet. The polyunsaturated fat in the American diet, e.g. use of canola oil that is preferred over olive oil, may have higher n-6 content.

Some limitations of our data and perhaps also our instrument are as follows. For most of the food components considered in our score, we did not account for overconsumption. This may have resulted in confounding by energy intake, as by

consuming higher amounts of foods, it may be easier to achieve recommended intakes, however also consequently more energy intake. One way to compensate for this limitation may be to adjust for total energy intake when analyzing these data in relation to disease outcomes. The inverse association observed with monounsaturated fats could be because in the traditional Mediterranean pyramid, olive oil is one of the important sources for monounsaturated fats. On the contrary in the US population where olive oil consumption is relatively low, much of the monounsaturated fats come from consumption of beef (58), which may reflect the inverse association seen with our instrument as higher quantity of red meat consumption received scores on the inverse ordinal scale. Finally the positive association seen with ω-6 fatty acids may be multifactorial, and could be a combination of not accounting for overconsumption of the food items, and may also reflect the inability of the semiquantitative FFQ to quantify nutrients accurately and could have affected the observed score-nutrient associations. Though importantly, both ω-6 and ω-3 fatty acids are essential for health and must be obtained from the diet, and our observed inverse association of (n-6):(n-3) fatty acid ratio is in the direction we would expect with adherence of Mediterranean dietary pattern.

In conclusion, in this study we developed a simple literature based Mediterranean diet score adapted to American diet, that shows reasonable associations with nutrient intakes. In addition, aMDS adherence was associated with a lower prevalence of chronic conditions including hypertension, diabetes mellitus and stroke. The Mediterranean diet has been globally recognized as a healthy diet. It makes sense to encourage US population to adapt the Mediterranean dietary patterns to prevent chronic diseases. This aMDS index could be used to assess adherence to Mediterranean diet in future studies.

Table 3.1 Components of the aMDS[1]

Components of the adapted[2] Mediterranean diet score	Scores[3] 0	1	2	3	4	5
Nuts	Never	< 1	1	2	3-4	≥ 5
Fruits	Never	1-4	5-8	9-15	16-21	≥ 22
Vegetables	Never	1-6	7-12	13-20	21-32	≥ 33
Fish	Never	< 1	1-2	3-4	5-6	≥ 7
Ratio of MUFAs to SFAs	1st sextile	Even percentile cut offs				6th Sextile
Non refined cereals	Never	1-6	7-12	13-18	19-31	≥31
Legumes	Never	< 1	1-2	3-4	5-6	≥ 7
Red meat and products	≥ 11	8-10	6-7	4-5	2-3	≤ 1
Poultry	≥ 11	9-10	7-8	5-6	4-5	≤ 3
Dairy products	≥ 31	29-30	21-28	16-20	11-15	≤ 10
Alcoholic beverages (g/day)						
Men	0 or > 70 gm	Even interval cut offs			> 0 and < 28 gm	
Women	0 or > 28 gm				> 0 and < 14 gm	

1 Values are frequencies in times/week for food components unless otherwise stated
2 Food items included apple, pears, orange, grape fruit, peaches, apricots, plums, banana, other fruits for fruits; cooked cereals such oatmeal grits and cream of wheat, other cereals, dark or whole grain breads, and corn bread for grains; beans, lentils, peanuts for legumes; carrots, broccoli, brussels sprouts, cauliflower, tomatoes, spinach, collard or other greens, green or lima beans, cabbage, cauliflower, squash, and any other vegetables for vegetables; clams, shrimp and any variety of fish for fish; milk (whole or regular and or low-fat milk), yogurt, ice cream, cheese, cheese dishes, and butter for dairy products; bacon, hamburgers, hotdogs and other processed meats such as sausage, salami, bologna etc., beef, pork, and ham for red meat and products; and chicken and turkey for poultry.
3 The top score contributes toward and the bottom score contributes against the Mediterranean diet. The total dietary score for an individual is the sum of individual scores for each of the 11 dietary components making up the aMDS. The possible overall aMDS ranged from 0 to 55, with higher values indicating greater adherence to the Mediterranean diet.

Table 3.2 Intake and score distributions of aMDS food components in the ARIC cohort

Components of the adapted Mediterranean diet score	Intake Distribution* Median (5^{th}, 95^{th} percentile)	Score Distribution Median (5^{th}, 95^{th} percentile)	Participants meeting recommended intake
Nuts	0.5 (0, 3)	1 (0, 4)	4.0 %
Fruits	10 (2.5, 23)	3 (1, 5)	12.5 %
Vegetables	11 (4, 22.5)	2 (1, 4)	2.8 %
Fish	1.5 (0, 4)	2 (0, 3)	4.3 %
Ratio of MUFAs to SFAs**	1.1 (0.9, 1.3)	3 (0, 5)	17.8 %
Non refined cereals (whole-grain)	8 (1.5, 21)	2 (1, 4)	1.0 %
Legumes	1.5 (0, 6)	2 (0, 4)	8.3 %
Red meat and products	6 (1.5, 13)	2 (0, 5)	12.2 %
Poultry	3 (0.5, 6)	4 (3-5)	55%
Dairy products	17 (6.5, 34)	3 (0 -5)	25.3 %
Alcohol intake (g/day)			
Male	0 (0, 40)	0 (0, 5)	24 %
Female	0 (0, 15)	0 (0, 5)	18.3 %

*Servings per week, unless otherwise specified; ** Ratio derived from the daily intake of MUFAs and SFAs

Table 3.3 Distribution of general and clinical characteristics of ARIC population according to quintiles of the adapted Mediterranean diet score

	Adapted Mediterranean diet score quintiles (range)					
Characteristics	Q1 (3-22) n=2,583	Q2 (22.1-25) n= 2,487	Q3 (25.1-28) n=2,471	Q4 (28.1-31) n=2,477	Q5 (31.1-49) n=2,377	*P-value*
Age (mean, SE)	59.9 (0.8)	59.8 (0.9)	59.8 (0.7)	60.1 (0.9)	60.2 (0.8)	0.72
Female %	56.3	58.8	57.0	54.3	49.7	0.10
Black Race (%)	19.7	22.4	22.1	22.7	23.8	0.05
College Educated (%)	42.1	451	45.9	49.4	53.1	
Current Smokers (%)	15	14	11	9	7	< 0.01
BMI	25.4 (1.8)	26.1 (1.5)	25.6 (1.4)	25.1 (1.9)	24.2 (1.6)	0.01
HTN	39.7	40.0	40.1	38.8	37.6	0.03
LDL (mean, SE)	126.6 (34.4)	126.8 (34.7)	128.03 (34.7)	127.2 (34.6)	126.7 (34.6)	0.56
Prevalent CAD	6.0	5.2	5.1	5.1	6.1	0.45
Prevalent DM	10.7	10.1	9.3	9.2	7.9	0.03
Prevalent Stroke	8.2	7.2	7.3	7.9	5.9	0.02

Table 3.4. Selected daily intakes of nutrients associated with the aMDS quintiles in the ARIC Cohort

Nutrients	**Adapted Mediterranean diet score quantiles (range)**					
	Q1 (3 – 22)	Q2 (22.1 – 25)	Q3 (25.1 – 28)	Q4 (28.1 – 31)	Q5 (31.1 – 49)	P-trend
n	2,583	2,487	2,471	2,477	2,377	
Energy intake, kJ	1,596	1,530	1,589	1,615	1,720	< 0.001
Fiber, g	13.5	15.8	17.8	19.3	23.4	< 0.001
Saturated fat, g	23.9	20.1	19.6	18.5	17.6	< 0.001
Trans fat, g	2.2	2.1	2.1	2.0	2.0	0.04
Monounsaturate	24.2	22.0	22.2	21.9	21.8	<

d fat, g						0.001
Oleic acid (18:1), g	22.1	20.0	20.3	20.0	19.9	< 0.001
Polyunsaturated fat, g	8.2	8.1	8.5	8.8	9.7	< 0.001
n-6 Fatty acids[1], g	7.1	7.0	7.3	7.5	8.2	< 0.001
n-3 Fatty acids[2], g	0.96	0.95	1.01	1.05	1.20	< 0.001
Ratio (n-6):(n-3) fatty acids	7.4	7.4	7.2	7.1	6.8	0.01
LA (18:2), g	7.0	6.8	7.2	7.4	8.1	< 0.001
ALA (18:3), g	0.8	0.7	0.7	0.7	0.6	0.01
Alcohol, g	4.2	4.1	4.7	5.7	6.6	< 0.001
EPA (20:5) + DHA (22:6), g	0.16	0.19	0.27	0.36	0.42	< 0.001
ß- Carotene, µg	2119.3	2781.3	3252.1	3611.4	4653.3	< 0.001
Lycopene, µg	846.3	946.3	1074.2	1202.9	1435.6	< 0.001
Folate, µg	216.1	238.8	257.7	274.1	322.7	< 0.001
Vitamin B6	1.7	1.8	1.9	2.0	2.2	< 0.001
Vitamin C, mg	109.5	123.8	130.7	139.5	163.7	< 0.001
Vitamin E, mg	4.1	4.6	5.1	5.6	6.8	< 0.001
Vitamin D, mg	250.9	240.2	254.3	260.1	302.1	0.01
Calcium, mg	738.6	654.9	644.8	655.6	643.6	0.01
Magnesium, mg	229.1	236.2	253.5	265.6	302.5	< 0.001
Potassium, mg	2479	2540	2685	2759	3094	< 0.001
hS-CRP (Mean,	3.0 (0.2)	2.8 (0.2)	2.7 (0.3)	2.5 (0.2)	2.2 (0.3)	<

SD)						0.001

LA: Linoleic acid; ALA: α-linolenic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid

[1] (n-6) Fatty acids included 18:2(n-6) (LA) and 20:4(n-6).

[2] (n-3) Fatty acids included 18:3(n-3) (LNA), 20:5(n-3) (EPA), and 22:6(n-3) (DHA).

CHAPTER 4

ASSOCIATION OF MEDITERRANEAN DIET ADHERENCE WITH INTRACRANIAL ATHEROSCLEROSIS, AND ITS ASSESSMENT OF EFFECT MODIFICATION BY INFLAMMATION

4.1 Introduction

Stroke is the third leading cause of death in the United States. Despite recent advances in acute stroke treatment, effective primary stroke prevention, by means of improved control of vascular risk factors, has the greatest potential to reduce its burden (59). Intracranial atherosclerotic disease (ICAD) of major cerebral vessels is a well-established vascular risk factors for ischemic stroke (60,61). The American Heart Association (AHA) has emphasized on the importance of a healthy diet for the primary and secondary prevention of cardiovascular diseases including stroke (62).

The western diet, rich in red meat, processed and artificially sweetened food products, refined grains, high fat-dairy products, and sugar-sweetened beverages, has been linked with increased levels of inflammatory biomarkers and increased risk for atherosclerotic disease (63). On the contrary, a Mediterranean dietary pattern, rich in nuts, olive oil, legumes and fish, fruits and vegetables, moderate intake of wine, has been

Current AHA guidelines specifically recommend Mediterranean-style diet as a lifestyle measure to reduce risk for clinical vascular events including stroke (62). Prior observational studies have shown inverse association between certain healthy eating

patterns and risk factors for atherosclerosis, including inflammatory biomarkers and endothelial function (67-70). Given the anti-inflammatory properties of Mediterranean diet (Med-Diet), at least in part the association between a Mediterranean diet and risk of stroke may be mediated by intracranial atherosclerotic disease (ICAD). However, to our knowledge the direct relationship between Med-Diet and ICAD has not been studied before.

The objective of our study was to examine the relationship between Mediterranean dietary pattern and ICAD in the Atherosclerotic Risk in Communities Study (ARIC). We hypothesized those who adherence to Mediterranean dietary pattern would be associated with lower burden of ICAD. We further assessed whether the association between Med-diet adherence and intracranial atherosclerotic disease is modified by inflammation. The examination of a Med-Diet in relation to both ICAD and inflammation, in a population-based US cohort makes the current study an important contribution to the literature on the potential benefits of a Med-Diet.

4.2 Methods

Study Population

The ARIC study is a community-based, prospective cohort total of 15,792 black and white participants, aged 45 to 64 years recruited in 1987 to 1989 randomly selected from 4 communities (Forsyth County, NC; Jackson, MS; suburbs of Minneapolis, MN; and Washington County, MD). Dietary intake was assessed by an interviewer-administered 66-item semi-quantitative food frequency questionnaire at baseline visit (1987–1989). Among 10,036 participants who were still alive at the time of study, 6538 (65%) took part in Visit 5. High-resolution magnetic resonance angiography (MRA) was offered to an age-stratified random sample of the participants from Visit 5 (n = 1,959), of

these 1,704 participants completed the study per study protocol with adequate image quality. From this sample, we excluded those who did not complete the baseline quantitative food frequency questionnaire (n = 259). We further excluded participants with missing information on Mediterranean dietary components (n=64) and nonwhite or black participants (n=3). After excluding these, a total of 1,378 participants met our inclusion criteria and their data were included in our analysis.

Assessment and classification of Intracranial Atherosclerosis

A sub cohort of ARIC participants who attended visit 5, completed high resolution MRA per study protocol. Details of MRA protocols, quality control, and reliability have been reported elsewhere (71). Briefly, all scans were performed on high resolution 3-Tesla Siemens MRI scanners. Images were analyzed by seven certified readers who were blinded on the information on participant characteristics. Percent inter and intra-reader agreement for ordinal intracranial stenosis were 94.4% and 93.8%, respectively. Intracranial vascular territories were categorized as intracranial internal carotid artery (cavernous and supraclinoid segments), middle cerebral artery (M1-M3 segments), anterior cerebral artery (A1-A3 segments), posterior cerebral artery (P1-P3 segments), basilar artery, and vertebral artery (V4 segment), for right and left hemisphere respectively. For each of the above intracranial vascular territory, the degree of stenosis was measured according to the criteria established in the Warfarin-Aspirin Symptomatic Intracranial Disease trial (i.e. no detectable stenosis, less than 50%, 51% to 70%, 71% to 99%, and complete occlusion) (72). For our primary analysis these categories were consolidated into 3 groups: no stenosis, < 50% stenosis, ≥ 50% stenosis/complete occlusion.

Assessment of Mediterranean dietary adherence

At baseline, ARIC participants completed a comprehensive interviewer-administered 66-item semi-quantitative food frequency questionnaire at baseline visit (1987–1989). This questionnaire was intended to represent typical food consumption over the previous year. Participants were asked to record how often each food was eaten.

We constructed an adapted Med-Diet score (aMDS) that has been adapted from proposed Med-Diet adherence scores as proposed by Trichopoulou et al (73) and Panagiotakos et al. (74). This score has been previously validated on ARIC cohort (Trivedi et al.). For the calculation of aMDS we included the following 11 food components: non-refined cereals (whole grains), fruits, vegetables (excluding potatoes), nuts, legumes, fish, monounsaturated-to-saturated fat ratio (as an alternative for olive oil), red and processed meats, dairy products, poultry and alcohol. We assigned scores in the range from 0 to 5 for the weekly consumption of each of these food components. Each component of the aMDS was calculated based on the updated recommended intakes of food in the Med-Diet pyramid (75). Items assumed to be contributing towards recommended Mediterranean dietary pattern received an incremental score ranging from 0 to 5, whereas consumption of food items against the Mediterranean dietary pattern received scores on the inverse ordinal scale.

The aMDS ranged from 0 to 55, with higher values indicating greater adherence to the Mediterranean diet. The aMDS, the primary exposure of interest, was analyzed by dividing the distribution score into Tertiles.

Covariates of interest

Age, gender, and race were reported by study participants at baseline. Cardiovascular risk factors included alcohol use (current, past, or never), smoking history (current, past, or never), prevalent hypertension, prevalent diabetes, cholesterol-lowering medication use, BMI categories (normal, overweight and obese). We defined hypertension as blood pressure of 140/90 mm Hg or higher, or use of antihypertensive medications; diabetes as fasting glucose level of ≥ 126 mg/dL or non-fasting glucose level ≥ 200 mg/dL, or use of antidiabetic medications; and dyslipidemia as total cholesterol level ≥ 240 mg/dL, low-density lipoprotein cholesterol level ≥ 160 mg/dL, high-density lipoprotein cholesterol level ≤ 40 mg/dL, or triglyceride level ≥ 200 mg/dL, or use of cholesterol-lowering medications. We categorized hs-CRP levels into two groups: low to moderate (hs-CRP ≤ 3 mg/l) and elevated (hs-CRP >3 mg/l).

Statistical analysis

The distribution of covariates of interest across aMDS Tertiles was examined using chi-square tests for categorical variables and analysis of variance for continuous variables. For the analyses aimed to assess the association between aMDS and ICAD, logistic regression models were constructed with ICAD as the dependent variable. We used a sequence of two models to assess the association between aMDS and levels of ICAD: 1) adjusted for age and sex; 2) adjusted for age, sex, race, education, prevalent diabetes, hypertension, hyperlipidemia and body mass index.

Lastly, we examined the effect for aMDS on ICAD is modified by inflammation (as measured by level of hs-CRP, as a dichotomous variable). For this analysis we categorized both aMDS and ICAD as a dichotomous variable: aMDS was categorized as

Adherent (above median score) vs. Non-adherent (below median score); ICAD as < 50% Stenosis vs ≥ 50% Stenosis.

For this, we calculated ORs for ICAD in each stratum of aMDS and hs-CRP, with the stratum with 3rd tertile of aMDS and hs-CRP < 3 mg/dl as the reference category. We calculated ORs for ICAD by Med-Diet adherence within strata of hs-CRP.

Finally, we presented measures of effect modification on both additive (relative excess risk due to interaction "RERI" [76,77]) and multiplicative scales with CIs and P-values.

We used SAS version 9.4 (SAS Institute Inc., Cary, NC) to conduct all statistical analyses. All statistical analyses were two-tailed, and a P value of 0.05 was considered statistically significant.

4.3 Results

Our analytic sample included 1,378 participants, who underwent MR Angiography and had diet data available to calculate Mediterranean diet score. The mean age of the participants was 59 ± 5 years, 60% were women, 75% were white, 47% attained higher than college education. For our study participants, aMDS ranged between 3 to 49. We categorized aMDS into Tertiles: Tertile 1 ranged from aMDS 3-23; Tertile 2 from aMDS 24-28; Tertile 3 from aMDS 29-49.

Characteristics of the study population stratified by aMDS tertiles are shown in Table 1. In the univariate analyses, male sex, those with higher than collage education were associated with greater adherence to Med-Diet pattern, while elevated BMI, hypertension, diabetes, dyslipidemia and diabetes were associated with lower consumption of a Mediterranean diet.

Table 2 shows the association between the aMDS Tertiles, and severe ICAS (≥ 50% stenosis). The prevalence of severe ICAS was higher in those with the lowest Tertile of aMDS (10.1 % for Tertile 1 vs. 9.7% for Tertile 2 vs. 9.4% for Tertile 3). In the adjusted logistic regression models, nonadherence to Med-Diet was associated with increased odds for severe ICAS (OR 1.27 and 1.19, for Tertile 1 and 2, respectively), however these results were not statistically significant.

We investigated whether the association of adherence to Mediterranean diet on ICAD was modified by the hs-CRP level. Hs-CRP was dichotomized as ≤ 3 mg/dl (low) or > 3 mg/dl (high). Odds ratios representing single and joint effects for Mediterranean diet (highest and lowest Tertile) and hs-CRP categories are presented in Table 4.3. Among individuals with high hs-CRP, non-adherence to Mediterranean diet was associated with higher odds for severe ICAS (aOR 1.45 [CI 0.876 – 2.3]), where aMDS adherence and low hs-CRP was the reference group. The RERI was calculated as 0.6 with a 95% CI obtained by the delta method (20) of (CI -0.12 - 0.94). The measure of interaction on a multiplicative scale, the ratio of ORs in strata of the hs-CRP, was 1.74 (0.68 – 3.20). Though not statistically significant, our findings were suggestive of positive effect modification on an additive and multiplicative scale. The relation between Med-Diet adherence and ICAD was adjusted for age, sex and race.

4.4 Discussion

The relation between Med-Diet and ICAD was not statistically significant in these analyses. However, our findings showed some suggestion that greater adherence to a Med-Diet may be associated with a decreased burden of severe ICAD. Possible explanations for not seeing a significant association could have been multifactorial. First,

between capturing diet and assessing ICAD there was a time difference of two decades, and individual's dietary habits could have been changed over this period for different reason. There have been many secular changes in American diet over this period. Factors like changes in national food supply, policy environment, scientific evidence and dietary recommendations may play a role (78). It is possible that with advancing age many participants may have changed their dietary pattern either owing to preference or because of competing risk factors (e.g. development of other chronic conditions), which could have led to misclassification of an individual's diet. Second, the dietary pattern of our cohort may not accurately reflect a truly Mediterranean diet. In reference to the recommended intake of individual components of Mediterranean diet, the consumption of vegetables, fruit, fish, and cereals was less in our cohort. Also this was less in reference to some other American cohorts which have assessed Mediterranean diet (79, 80, 81). In comparison to traditional Mediterranean diet, we also observed variations in some derived nutrients. For instance, we saw an inverse association for MUFAs, which are mostly derived from olive oil, then expected in Mediterranean diet. Overall, the dietary habits of our cohort at baseline were less consistent with a Mediterranean-style pattern.

Multiple risk factors for ICAD, including smoking, hypertension, diabetes have been previously reported. Med-Diet have been linked with extracranial atherosclerosis including coronary artery disease (84,85) and cervical carotid stenosis (86,87), however no prior study to our knowledge has specifically studied diet as a risk factor for intracranial atherosclerosis. Although extracranial atherosclerosis and intracranial atherosclerosis share multiple common risk factors, differences still exist between them (88,89). For example male sex and hyperlipidemia have been shown to

tend to be more important in extracranial atherosclerosis than ICAD, while metabolic syndrome favors posterior circulation of the intracranial vessels (88). Different vessel wall structure and hemodynamics of intracranial vs. extracranial arteries may partly explain some of the observed differences, as this may vary the response to various pathophysiological processes [88,90]. Thus, it is important to identify modifiable risk factors specific for intracranial atherosclerosis, like diet, that can be used as a management strategy to address ICAD among high risk populations.

The cardiovascular protective effects of Med-Diet are multifactorial. Some of the well-studied mechanisms include reduced inflammation, decreased platelet aggregation, reduced oxidative stress, improved lipid profile, improved blood pressure, and weight reduction. Clearly no mechanism can independently account for observed cardiovascular benefits by itself, and there is often a complex interplay between these mediators.

Our findings suggest, inflammation modifies the observed association between Med-Diet and ICAD. Nonadherence to Med-Diet was associated with an increased burden of ICAD, however this association was only significant in those with high hs-CRP levels. The combined effect of Med-Diet and hs-CRP was a little larger than sum of their individual effects suggesting a possible synergistic interaction. This could imply that certain at-risk population with a high hs-CRP level, adherence to a Mediterranean pattern diet can reduce risk for intracranial atherosclerotic disease, and thus eventually ischemic strokes.

The strengths of our study include (1) it is large population-based cohort, with high rate of patient follow up within the ARIC cohort; (2) dietary data and vascular risk factors were rigorously measured as a part of the ARIC cohort, (3) the availability of high resolution MR Angiography data to assess the degree of ICAD, (4) MR Angiography protocols and interpretation was standardized across centers.

However, our study also had some limitations. First, since we did not have serial ICAD measurements, our analysis is partially cross-sectional, and any assumption on causality of the observed association would be subject to bias. Second, since we only have baseline data on diet, it may not reflect lifelong dietary habits of participants. Third, it is possible that some participants change their dietary habits and switch towards an unhealthy dietary pattern as they age, resulting in misclassification and underestimation of the effect size. Reverse causation is also possible, as those with underlying risk factors may modify their diet to a healthier pattern.

In conclusion, our study is the first large observational population-based study to examine the relationship between Med-Diet and ICAD. Our findings suggest that dietary pattern consistent with a Med-Diet may have some benefits to reduce burden of ICAD, an important risk factor for stroke. The benefits of Med-Diet may even be higher in those with underlying increased inflammatory states. Investigations using prospective study designs are needed to assess how adherence to a Med-Diet may impact the trajectories of markers of inflammation, which modify the risk of intracranial atherosclerosis and vascular outcomes, over time. Further research on the role of diet on ICAD should be a public health priority because it is a modifiable behavioral risk factor.

Table 4.1 Selected characteristics of the study population by Tertiles of Mediterranean diet score (n=1,378)

	Cohort (n=1,378)	First Tertile (n=454)	Second Tertile (n=476)	Third Tertile (n= 446)
	Mean (SD) or %	MD Score 3 - 24	MD Score 25 - 28	MD Score 29 - 49
Age	58.6 (5.1)	58.4 (5.1)	58.5 (5.6)	59.1 (5.3)
Female (%)	59.5 %	62.9 %	60.4 %	53.9 %
Race – Black (%)	25.1 %	22.9 %	23.5 %	22.2 %
Education				
<High School	12.3 %	18.5 %	11.4 %	8.9 %
High School or College	40.6 %	43.4 %	41.3 %	37.8 %
>College	47.1 %	38.2 %	47.3 %	53.3 %
Body Mass Index†	26.8 (4.1)	27.2 (4.7)	26.8 (4.5)	26.32(4.1)
Hypertension (%)	31.4 %	37.5 %	29.4 %	27.1 %
Diabetes (%)	7.5 %	8.2 %	7.4 %	7.2 %
Dyslipidemia	17.3 %	20.0 %	17.2 %	15.0 %
Current Smoker (%)	9.5 %	11.39%	11.9 %	6.0%

Table 4.2 Logistic regression models with Odds Ratios for ICAS categories by aMDS Tertiles

	Severe ICAS (≥ 50%)		
	Cases / Total N (%)	Odds Ratio (Crude Model*)	Odds Ratio (Adjusted model**)
aMDS Tertile 1	46/454 (10.1%)	1.33 (0.80 - 2.22)	1.27 (0.78 - 2.09)
aMDS Tertile 2	46/476 (9.7%)	1.28 (0.81 - 2.20)	1.19 (0.75- 2.07)
aMDS Tertile 3	42/446 (9.4%)	Reference	Reference
***AUC		0.68	0.71

*Crude logistic regression models adjusted for age and sex only.
** Logistic regression models were adjusted for the following covariates: age, sex, race, education, prevalent diabetes, hypertension, hyperlipidemia, and body mass index.
***Area Under Curve

Table 4. 3 Odds ratios representing single and joint effects for Mediterranean diet (highest and lowest Tertile) and hs-CRP categories.

	Med-Diet Adherence*		Med-Diet Nonadherence*		
	N with/without ICAS	OR (95% CI)	N with/without ICAS	OR (95% CI)	OR (95% CI) for ICAD by Med-Diet non-adherence within strata of hs-CRP
Hs-CRP ≤ 3	26/258	1	18/241	0.78 (0.42 – 1.28); P=0.10	0.78 (0.42 – 1.28); P=0.10
Hs-CRP > 3	16/146	1.07 (0.59 – 1.95)	26/169	1.45 (0.87-2.36); P=0.09	1.35 (0.75 – 2.42); P=0.47
Measure of effect modification on additive scale: RERI (90% CI) 0.6 (-0.12 - 0.94); P=0.22; Measure of effect modification on multiplicative scale: ratio of 1.74 (0.68 – 3.20); P=0.43; ORs are adjusted for age and sex, race					

CHAPTER 5

SUMMARY

Does inflammation play a role in ICAS? Is it an important driving force in ICAS or just an epiphenomenon? Besides optimal medical management is there a space for targeted lifestyle interventions such as diet in the management of ICAS? These were few of the questions we sought to understand in this research.

Atherosclerotic stenosis of intracranial vessels as a disease has a complex genesis. Based on the current understanding of the risk factors for ICAS, targeting blood pressure, cholesterol and cigarette smoking has remained the corner stone for the medical management of ICAS and secondary stroke prevention. Although clinical and observational studies have provided evidence for role of inflammation, at least in the extracranial atherosclerotic disease and cardiovascular prevention, many clinicians do not see any reason to consider screening and managing inflammation as part of the preventive strategy, nor does this reflect in current guidelines for stroke prevention. The results from our study demand a reassessment of the situation.

Our findings show inflammation plays a key role in stenosis of major intracranial vessels. More specifically, chronically high level of inflammation, as assessed through repeated measurements of hs-CRP was found to be associated with ICAS. These findings support prior evidence that chronically high inflammation play an active role in inflammation has been linked with cardiovascular diseases including ischemic stroke, it is

likely ICAS may be playing a part on a causal pathway, as an intermediate risk factor linking inflammation and ischemic stroke. Our findings suggest that greater adherence to a Mediterranean dietary pattern may have some benefits in prevention of ICAS. The cardiovascular protective effects of Mediterranean diet could be multifactorial. Some of the well-studied mechanisms include reduced inflammation, reduced oxidative stress, improved lipid profile, improved blood pressure, and weight reduction. Furthermore, our analyses indicates inflammation modifies the observed association between Mediterranean diet and ICAS, whereas nonadherence to Mediterranean appears to be associated with an increased burden of ICAD, however this association was only meaningful in those with high hs-CRP level. It implies that in those with chronic inflammation, adherence to a Mediterranean pattern diet can reduce risk for intracranial atherosclerotic disease, and thus eventually ischemic strokes.

It is important to improve our understanding on the role of inflammation as a risk for atherosclerotic disease. Among population at risk, such as those suffering with ischemic stroke, it is important to screen for elevated hs-CRP levels for those with ICAS, and vice-versa. Current clinical practice guidelines do not recommend screening for inflammation for those with symptomatic or asymptomatic ICAS, however this should be a consideration.

While considering risk factor stratification for secondary stroke prevention in population with known ICAS, it is also important to monitor hs-CRP levels, and pursue targeted treatments to address chronic inflammatory states. We have limited evidence on the benefits of managing inflammation with pharmacological agents for primary and

secondary cardiovascular prevention. Investigations using prospective study designs are needed to assess how healthy lifestyle changes such as adherence to a Mediterranean

Dietary pattern may impact the trajectories of markers of inflammation, which can modify the risk of intracranial atherosclerosis and vascular outcomes, over time. Further research on the role of inflammation on vascular intermediate risk factors such as ICAD, and understanding its modifiable behavioral risk factors such as diet should be a public health priority.

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