Lifestyle Factors During the Midlife and Subclinical Carotid Atherosclerosis Later in Life:

The Study of Women's Health Across the Nation

by

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List of Abbreviations

AD	Adventitial diameter
AHEI	Alternate Healthy Eating Index
AMS	Artery Measurement Software
ASB	Artificially sweetened beverages
BMI	Body mass index
CCA	Common carotid artery
CES-D	Center for Epidemiologic Studies Depression scale
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
FFQ	Food frequency questionnaire
HDL	High-density lipoprotein
HHS	United States Department of Health and Human Services
HLS	Healthy Lifestyle Score
IMT	Intima-media thickness
LDL	Low-density lipoprotein
LS-mean	Least squares mean
MSM	Marginal structural model
NHLBI	National Heart, Lung, and Blood Institute

NHS	Nurses' Health Study
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor 1
PCA	Principal component analysis
PLS	Partial least squares regression
P:S	Ratio of polyunsaturated to saturated fatty acids
PUFA	Polyunsaturated fatty acids
RR	Risk ratio
RRR	Reduced rank regression
SD	Standard deviation
SFA	Saturated fatty acids
SSB	Sugar-sweetened beverages
SWAN	Study of Women's Health Across the Nation
U.S.	United States
USDA	United States Department of Agriculture

Abstract

Menopause has adverse effects on cardiometabolic profiles that are linked to an accelerated progression of atherosclerosis and increased risk of cardiovascular disease in women. Thus, the menopausal transition may be a critical window for cardiovascular prevention in women, and lifestyle improvements during the menopausal transition may counteract the menopause-induced atherosclerotic risk. However, the potential impacts of lifestyle behaviors during the midlife on atherosclerosis later in life among women are still unclear.

This dissertation evaluates the prospective associations of various lifestyle factors during the midlife with subclinical carotid atherosclerosis later in women's life. Data from the Study of Women's Health Across the Nation were used. Repeatedly collected lifestyle-related exposures, including dietary intake, smoking status, and physical activity, were available over 10 years. The measures of subclinical carotid atherosclerosis included common carotid artery intima-media thickness, adventitial diameter, and carotid plaque, all collected approximately 14 years after the baseline.

In Chapter 2, the prospective associations between the intakes of eight beverage groups (coffee, tea, sugar-sweetened beverages, artificially sweetened beverages, fruit juices, whole milk, milk with lower fat content, and alcoholic beverages) during the midlife and subclinical carotid atherosclerosis were examined. A total of 931 midlife women were included in this analysis. The main findings indicated that occasional coffee intake during the midlife (no more than 2 cups per day) was positively associated with intima-media thickness later in life whereas moderate-to-heavy intake (more than 4 cups per day) might be inversely associated with intima-

media thickness. Further, moderate intake of alcoholic beverages was inversely associated with intima-media thickness.

In Chapter 3, the prospective associations of empirically derived dietary patterns with subclinical carotid atherosclerosis were assessed. A total of 1,246 midlife women were included in this analysis. Three statistical methods, including principal component analysis, reduced rank regression, and partial least squares regression, were used to identify dietary patterns. A Western dietary pattern was identified from each method. Further, a positive association between the Western diet and intima-media thickness was found under all three statistical methods. The findings suggested that the adoption of a diet low in red meat, processed meat, deep fried products, and sugar-sweetened beverages during the midlife might protect against future atherosclerosis in women.

In Chapter 4, a 10-year average midlife Healthy Lifestyle Score was constructed using data on smoking, diet, and physical activity from 1,143 women. The prospective association between the Healthy Lifestyle Score and subclinical carotid atherosclerosis was evaluated. The prevalences of healthy behaviors were extremely low in midlife women. Further, women who had a healthy lifestyle during the midlife, composed of abstinence from smoking, having a healthy diet, and engagement in regular physical activity, had less subclinical carotid atherosclerosis later in their life. Among the three components of the Healthy Lifestyle Score, abstinence from smoking had the strongest association with subclinical carotid atherosclerosis.

This dissertation highlights the midlife as a pivotal period for cardiovascular prevention in women and suggests that primary prevention efforts should focus on modifiable behaviors including diet, smoking, and physical activity. In the final chapter, the public health implications of the findings are discussed, and potential future research directions are reviewed.

Chapter 1. Introduction

1.1 Cardiovascular Disease and Subclinical Atherosclerosis in Midlife Women

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States and globally. Although mortality attributable to CVD has been declining in the U.S. due to improved treatments, CVD currently still claims more lives than cancer and chronic lower respiratory disease combined.[1] It is projected that, by the year 2035, 45% of the U.S. adults would have some form of CVD (including heart disease, stroke, hypertension, peripheral artery disease, and diseases of the veins), and the total yearly cost of care for CVD in the U.S. would reach $1.1 trillion.[1]

CVD is the most common disease and the top killer of American women. About 1 in 3 U.S. women have some form of CVD, and nearly half of women's deaths after age 50 are due to CVD.[1] Importantly, women experience a steeper increase in cardiovascular risk after the menopausal transition relative to before the menopause.[1-3] Menopause is also associated with a series of unfavorable changes in cardiometabolic risk factors independently of chronological aging, such as increases in total abdominal fat and visceral fat,[4, 5] increased levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and apolipoprotein B,[6] and reduced cardioprotective effect of high-density lipoprotein (HDL) cholesterol or even a reversal of its effect.[7, 8]

Subclinical atherosclerosis is the systemic process in which fatty deposits, inflammatory cells, and scar tissue build up within the walls of arteries before the manifestation of any clinical

symptoms.[1] It typically precedes the occurrence of clinical cardiovascular events by years or even decades.[9] Markers of subclinical carotid atherosclerosis, usually measured at the common carotid artery (CCA), include intima-media thickness (IMT), adventitial diameter (AD), and plaque formation. IMT is an early marker of atherosclerosis and reflects the thickening of the vessel wall due to chronic inflammation and lipid deposition.[10] An elevated AD is a marker of vascular remodeling and represents dilation of the artery, disturbance in blood flow, and reduced arterial flexibility.[11] Finally, the development of a plaque is the hallmark of atherosclerosis that results from the prolonged accumulation of inflammation, deposition, and calcification.[10]

Accumulating evidence suggests that the measures of subclinical carotid atherosclerosis are important predictors of CVD events later in life,[12, 13] and can be used to quantify the cardiovascular risk in asymptomatic individuals.[12-14] In a meta-analysis, Lorenz et al. reported that, after adjusting for age and sex, every increase of one standard deviation (SD) in CCA-IMT was associated with a 26% higher risk of myocardial infarction with a 95% confidence interval (CI) of 21% to 30%, and a 32% higher risk of stroke (95% CI: 27%, 38%).[15] Similarly, data from the Atherosclerosis Risk in Communities Study suggested that every one-SD increase in CCA-AD was associated with an 18% higher hazard of incident cardiac events (95% CI: 0%, 40%) and 73% higher prevalence of myocardial infarction (95% CI: 37%, 117%), even after adjusting for known CVD risk factors and CCA-IMT.[16] Likewise, the presence of carotid plaque is associated with 83%, 210%, and 81%-196% higher hazard of myocardial infarction,[17] stroke,[18] and death from coronary heart disease,[19, 20] respectively.

In addition to being predictive of clinical CVD, the extent of carotid atherosclerosis is also associated with an accelerated decline in physical and cognitive functioning even among those without clinical CVD or after accounting for the intervening clinical CVD outcomes.[21-24]

Of note, it has been estimated that, among women aged 65 years and older, those with subclinical atherosclerosis had a trajectory of decline in physical and cognitive functioning equivalent to those who were 6.5 years older but without subclinical atherosclerosis.[21] Therefore, subclinical atherosclerosis is not merely a precursor to CVD, but an intermediate endpoint that is worth examining in its own right.

The distribution and determinants of subclinical atherosclerosis vary appreciably by age and sex.[25] The menopausal transition in women is associated with an accelerated progression of carotid atherosclerosis.[11, 26] The progression rate of CCA-IMT increased significantly from 0.005-0.007 mm/year during the premenopausal and the early peri-menopausal stages to 0.017 mm/year during the late peri-menopausal stage. Similarly, the progression of CCA-AD changed significantly from −0.032 mm/year during the premenopausal stage to 0.024 mm/year in the late peri-menopausal stage and 0.018 mm/year in the postmenopausal stage.[11]

To summarize, the midlife, which is typically defined as age 40 to 65 years and includes the menopausal transition,[27] represents a crucial window for cardiovascular risk assessment and prevention among women. The menopause-induced cardiovascular risk may be counteracted by improvements in lifestyle factors during the menopausal transition. However, few prior studies have evaluated the potential effects of lifestyle factors during the midlife on cardiovascular risk such as subclinical atherosclerosis later in life among women.

1.2 Beverage Intake and Subclinical Atherosclerosis

Beverages are not always considered or remembered when individuals think about their diets. However, beverage is an essential component of dietary intake and has crucial public-health implications. An average American consumed 135 gallons of beverages other than water

every year.[28] In the U.S., the consumption of healthful beverages is low, whereas the consumption of unhealthy beverages is high.[28] Most types of beverages consumed in the U.S. are energy-dense and may result in considerably higher total energy intake.[28] Accumulating evidence suggests that the consumption of energy-dense beverages promotes the development of hypertension, hypertriglyceridemia, and clinical coronary heart disease.[29] Increased intake of energy-dense beverages is suspected to be one of the major contributors to the current obesity epidemic in the U.S.[29] Furthermore, numerous compounds present in commonly consumed beverages, such as caffeine[30] in coffee, and polyphenolic constituents in coffee[31-36] and tea[32, 37-39] may have preventive or detrimental effects on cardiometabolic health.

While the potential effects of specific nutrients on cardiovascular health are frequently evaluated in nutritional epidemiologic studies, the impacts of beverage intake on subclinical atherosclerosis are relatively less understood. The existing literature regarding the effects of coffee,[40-44] tea,[41, 43, 45, 46] and milk[47, 48] on subclinical atherosclerosis is inconsistent. Prior studies on the intake of alcoholic beverages and subclinical atherosclerosis also have conflicting results, with some studies reporting a J-shaped pattern in women and men,[49-51] some finding an association in men but not in women,[52, 53] and some failing to detect an association at all.[54-56] Literature on sugar-sweetened beverages (SSB), artificially sweetened beverages (ASB), and fruit juices on subclinical atherosclerosis are scarce. To date, no study has specifically examined the prospective association between the beverage intake in midlife women and subclinical atherosclerosis later in their life.

1.3 Dietary Patterns and Subclinical Atherosclerosis

Diet is one of the most important modifiable risk factors for CVD. The traditional approach in nutritional epidemiology focuses on the effects of individual foods and nutrients. Over the past two decades, dietary pattern analysis has gained popularity as a promising alternative approach to investigating the effect of the overall diet. Dietary pattern analysis is less prone to some methodological limitations of the traditional "single food/nutrient" approach, such as small effects of individual foods, complicated interaction among nutrients, and mutual confounding between dietary exposures.[57, 58] Results from dietary pattern analyses can also be more directly transformed into dietary guidelines as they focus on whole foods instead of nutrient components. Existing evidence suggests that the "Western" dietary pattern, characterized by high intakes of red meat, processed meat, sweets, desserts, refined grains, sugar-sweetened beverages, and deep fried products, may be positively associated with the risk of CVD incidence and mortality.[57] On the other hand, the "Prudent" dietary pattern, characterized by high intakes of fruits, vegetables, legumes, nuts, fish, poultry, and whole grains, may be associated with a lower risk of CVD.[57]

Compared to evidence for clinical CVD, the literature on diet and subclinical atherosclerosis is limited and inconsistent, especially for midlife women undergoing the menopausal transition and for women in minority racial/ethnic groups. Some studies suggested that adherence to specific dietary patterns may be associated with subclinical atherosclerosis,[59-63] whereas many others found no evidence for an association.[64-67] The inconsistent results from previous studies may be related to the differences in study design, study population, as well as the specific vascular beds used to measure atherosclerosis (e.g., aorta, coronary, or carotid). However, the mixed results in the literature may also be partially attributable to the measurement

error of long-term diet. Most prior studies used dietary data collected at a single time point to derive dietary patterns. However, as diets may change over time, only one dietary measurement may not accurately capture the long-term dietary practices.[68, 69] There is evidence that the dietary patterns among midlife women may become unstable after 6 to 7 years.[70] Therefore, when the follow-up period is long and when the long-term effect of diet is of interest, using the cumulative dietary exposures from repeated measures is preferable and may yield stronger associations with CVD outcomes than using either only the baseline diet or the most recent diet.[68, 69] Furthermore, multiple variable reduction techniques exist for empirical dietary pattern analyses, which can also lead to inconsistent results, as further discussed in the next section.

1.4 Analytical Approaches to Identifying Empirical Dietary Patterns

Principal component analysis (PCA) is one of the most commonly used methods for empirical dietary pattern analysis. PCA combines correlated food items into weighted linear combinations that account for maximum variation of the original food variables.[71] A limitation of PCA is that the derived dietary patterns are based solely on the statistical correlations among the food variables (i.e., which foods are commonly consumed together). As a result, PCA-derived patterns may not necessarily explain a meaningful amount of variation in nutrient intake or other biological mediators, thus may not identify the food combinations that are most relevant to disease outcomes.[71-73] Also, PCA is a purely data-driven approach that does not allow for the incorporation of any *a priori* knowledge of the potential biological mechanisms of the diet-disease associations.

Reduced rank regression (RRR) and partial least squares regression (PLS) are alternative variable reduction techniques.[71, 74, 75] As opposed to PCA, these alternative methods combined *a*

posteriori analysis with *a priori* knowledge and allow for the incorporation of intermediate response variables in the identification of dietary patterns. The RRR aims to identify dietary patterns that explain the maximum variation in a set of prespecified intermediate response variables (as opposed to the maximum variation in the food variables themselves in PCA). The intermediate response variables are nutrients or biomarkers that are known to impact or predict the disease. For example, in a population-based case-control study of 455 midlife or elderly women in Germany, Hoffmann et al. used five well-established serum biomarkers of coronary artery disease [LDL cholesterol, HDL cholesterol, lipoprotein(a), C-reactive protein, and C-peptide] as the intermediate response variables in RRR. They found that a pattern resembling a Western diet explained the maximum variation in the intermediate response variables, and was also strongly associated with higher odds of coronary artery disease.[72] The PLS is a compromise between PCA and RRR; PLS-derived dietary patterns aim to explain variation in both the intermediate response variables and the food variables. Due to the incorporation of the intermediate response variables, RRR and PLS can not only evaluate diet-disease associations but also assess the potential biological mechanisms responsible for the observed associations. Despite the methodological attractiveness of the alternative techniques, the performances of PCA, RRR, and PLS in the estimation of diet-disease associations have rarely been compared.[75-77]

1.5 Overall Healthy Lifestyle and Subclinical Atherosclerosis

Smoking,[78] unhealthy diet,[58] and lack of regular physical activity[79] are well-known modifiable behavioral risk factors for CVD. Prior studies have found inverse associations between the overall healthy lifestyle and various CVD outcomes, including coronary heart

disease,[80] myocardial infarction,[81] and CVD-related mortality.[82] The American Heart Association's Strategic Impact Goals aim to improve the cardiovascular health of all Americans by 20% while reducing deaths from cardiovascular disease and stroke by 20% by 2020 through changes in these critical lifestyle factors.[9]

A composite healthy lifestyle index constructed by individual risk factors can provide unique information on the effects of overall lifestyle. Notably, in a landmark study, Stampfer et al. followed 84,129 midlife and older women enrolled in the Nurses' Health Study (NHS) for 14 years, and found that women who did not smoke, who engaged in moderate-to-vigorous physical activity for ≥ 30 minutes/day, and who had a healthy diet (scoring in the upper 40% for a diet high in cereal fiber, marine n-3 fatty acids, folate, the ratio of polyunsaturated to saturated fatty acids, and low in trans fat and glycemic load) had 57% lower odds of coronary events compared with all the other women who did not have this lifestyle.[80] A recent analysis in the NHS also found that women with a low-risk lifestyle (never smoking, normal BMI, moderate-to-vigorous physical activity, moderate alcohol intake, and high diet quality) had 74% lower hazard of all-cause mortality and 82% lower hazard of CVD mortality, compared to those with none of the low-risk factors.[83] The projected life expectancy at age 50 years comparing women with five low-risk factors with women with none was 14 years (95% CI: 11.8, 16.2) longer, 30.8% of which was attributable to fewer CVD death.[83]

The potential effect of a healthy lifestyle on the extent of subclinical atherosclerosis has been evaluated in few studies. Moreover, no study has examined the prospective association between an overall healthy lifestyle index in women during the midlife and subclinical atherosclerosis later in life. The menopausal transition is associated with an accelerated progression of subclinical carotid atherosclerosis,[11, 26] and lifestyle intervention during this life

stage may slow the menopause-related progression of atherosclerosis.[26] Therefore, the impact of a healthy midlife lifestyle on subclinical atherosclerosis warrants further investigations.

1.6 Specific Aims and Hypotheses

Aim 1

To evaluate the prospective associations between the average intakes of eight groups of beverages during the midlife and subclinical carotid atherosclerosis later in life.

Hypothesis 1a: Intakes of coffee and tea would be inversely associated with subclinical carotid atherosclerosis.

Hypothesis 1b: Intake of sugar-sweetened beverages would be positively associated with subclinical carotid atherosclerosis.

Hypothesis 1c: Intake of alcoholic beverages would be associated with subclinical carotid atherosclerosis in a J-shaped pattern.

Hypothesis 1d: Intakes of artificially sweetened beverages, fruit juices, whole milk, and milk with lower fat content would not be associated with subclinical carotid atherosclerosis.

Aim 2

To identify empirical dietary patterns in midlife women using three complementary variable reduction techniques, including principal component analysis (PCA), reduced rank regression (RRR), and partial least squares regression (PLS), and to evaluate the prospective associations between the identified patterns and subclinical carotid atherosclerosis. We also aimed to compare the performances of the three variable reduction techniques.

Hypothesis 2a: At least two dietary patterns would be identified from PCA, including a "Western" pattern and a "Prudent" pattern. Adherence to the "Western" pattern would be positively associated with subclinical carotid atherosclerosis, whereas adherence to the "Prudent" pattern would be inversely associated with subclinical carotid atherosclerosis.

Hypothesis 2b: The patterns identified from RRR and PLS would be more strongly associated with subclinical carotid atherosclerosis compared to the PCA-derived patterns due to the incorporation of intermediate response variables.

Aim 3

To construct a composite midlife Healthy Lifestyle Score using smoking status, diet quality, and physical activity, and to evaluate the prospective associations of the midlife Healthy Lifestyle Score and its three individual components with subclinical carotid atherosclerosis later in life.

Hypothesis 3: The Healthy Lifestyle Score would be inversely associated with subclinical carotid atherosclerosis.

1.7 Summary of Chapters

This dissertation aimed to examine the impacts of various lifestyle factors during the midlife on subclinical atherosclerosis later in life among women. Chapters 2, 3, and 4 correspond to the Aims 1, 2, and 3 as mentioned earlier, and used beverage intake, dietary patterns, and healthy lifestyle as the main exposures, respectively. The three groups of exposures represented the three levels of investigations in nutritional/chronic disease epidemiology: single food (Chapter 2), whole diet (Chapter 3), and overall lifestyle (Chapter 4). We aimed to provide insights into potential dietary and lifestyle interventions targeted toward midlife women for

healthy aging and primary prevention of cardiovascular disease. Additionally, from a methodological standpoint, we also aimed to use Chapter 3 to contribute new information on the application of multiple variable reduction techniques in nutritional epidemiological research.

Chapter 2. Prospective Associations Between Beverage Intake During the Midlife and Subclinical Carotid Atherosclerosis

Submitted for publication in peer-reviewed journal

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2.1 Abstract

Background: Beverage intake may have impacts on the atherosclerotic process. The menopausal transition is a critical window for cardiovascular prevention in women, but the potential effect of beverage intake during the midlife on future subclinical atherosclerosis among women is unclear. **Methods:** The prospective associations between the intakes of eight beverage groups (coffee, tea, sugar-sweetened beverages, artificially sweetened beverages, fruit juices, whole milk, milk with lower fat content, and alcoholic beverages) during the midlife and subclinical carotid atherosclerosis were examined. A total of 931 midlife women had data available on average beverage intake and measures of carotid atherosclerosis and remodeling including common carotid artery intima-media thickness (CCA-IMT), adventitial diameter (CCA-AD), and carotid plaque index, collected 14 years later. The associations of beverage intake with CCA-IMT and CCA-AD were estimated using linear models; the associations between beverage intake and carotid plaque were estimated using log-binomial models.

Results: Coffee intake was associated with CCA-IMT in an inverted J-shaped pattern (*P*-curve = 0.004). Women who consumed >0 to 1 cup, >1 to 2 cups, >2 to 4 cups, and >4 cups of coffee per day had 0.031 mm larger (95% CI: 0.008, 0.054), 0.026 mm larger (95% CI: 0, 0.051), 0.009 mm larger (95% CI: -0.018, 0.036), and 0.029 mm smaller (95% CI: -0.073, 0.015) CCA-IMT, respectively, compared to coffee non-drinkers. There was an inverse linear association between alcoholic beverage intake and CCA-IMT (*P*-trend = 0.009); every one-serving per day increase associated with a 0.019 mm smaller CCA-IMT (95% CI: -0.032, -0.005). No significant associations were observed for other beverage groups.

Conclusions: Coffee intake during the midlife in women is associated with a larger CCA-IMT in the future except among women who consumed more than 4 cups of coffee per day. Moderate alcohol intake is associated with a smaller CCA-IMT later in life.

2.2 Introduction

In the United States, cardiovascular disease (CVD) remains the leading cause of mortality and morbidity in women.[1] Although deaths attributable to CVD have been declining, CVD currently still claims more lives than cancer and chronic lower respiratory disease combined.[1] It is estimated that 45.1% of the American adults will have some form of CVD by 2035.[1] Women's risk of CVD increases after the menopausal transition.[1] Menopause is associated with several adverse changes in cardiovascular risk factors, such as increased visceral adiposity[4] and elevated levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and apolipoprotein B,[6] independently of chronological aging.

Measures of subclinical atherosclerosis and vascular remodeling of the carotid arteries are clinically important predictors of future CVD events.[13] Furthermore, they are associated with poorer physical and cognitive functioning in old age independent of clinical CVD.[21-23] In women, accelerated progression of subclinical carotid atherosclerosis occurs during the menopausal transition.[11, 26] Therefore, the midlife, which includes the menopausal transition, may be a crucial period for cardiovascular risk prevention among women.

Beverage intake is a major component of people's diets and is of high public-health importance. An average American consumes 1.5 liters per day, or 135 gallons per year, of beverages other than water.[28] In the U.S., consumption of unhealthy beverages (e.g., sugar-sweetened beverages) is high, whereas consumption of beverages considered healthful (e.g., low-fat milk and tea) is falling.[28] Numerous compounds present in some beverages, such as caffeine[30] in coffee, and phenolic constituents in coffee[31-36] and tea[32, 37-39] may delay or accelerate the atherosclerotic process. Furthermore, most types of beverages consumed in the U.S. contain calories. It is known that intake of energy-dense beverages cannot satisfy the appetite and the

human body cannot correspondingly adjust the energy intake from solid foods to compensate for the extra calories from those beverages.[28] Increased intake of energy-dense beverages is one of the major contributors to the current obesity epidemic in America.[29] Low-calorie, artificially sweetened beverages have been proposed as a potential replacement for sugar-sweetened beverages, but there is scant evidence on the adverse effects of artificially sweetened beverages relative to their potential benefits.[84] Intake of alcoholic beverages has been associated with multiple cardiovascular outcomes in a J-shaped pattern, with the lowest risk observed for people who had light to moderate levels of consumption.[85] A recent pooled analysis using data from 83 prospective cohort studies, however, suggested that the optimal levels of alcohol consumption for CVD prevention may be lower than those recommended in most current guidelines.[86]

The existing body of evidence regarding the effects of beverage intake on CVD is inconsistent, and few studies have examined the prospective association between beverage intake in midlife women and subclinical atherosclerosis later in life. Therefore, we aimed to use data from the Study of Women's Health Across the Nation (SWAN) to evaluate the prospective associations between the intakes of eight groups of beverages during the midlife and measures of subclinical carotid atherosclerosis.

2.3 Methods

2.3.1 Study Design and Study Population

The SWAN is an ongoing, multicenter, multiethnic, prospective cohort study initiated in 1996 to study the natural history of the menopausal transition. Details of the SWAN protocol have been described previously.[87] Briefly, SWAN participants were recruited from seven sites across the U.S.: 1) Boston, Massachusetts; 2) Chicago, Illinois; 3) Southeastern Michigan; 4) Los Angeles, California; 5) Newark, New Jersey; 6) Pittsburgh, Pennsylvania; and 7) Oakland, California. At baseline, 3,302 women who self-identified as African American (Pittsburgh, Chicago, Detroit, and Boston), Chinese (Oakland), Japanese (Los Angeles), Hispanic (Newark), or non-Hispanic white (all sites) were enrolled. Baseline eligibility criteria included age 42 to 52 years, having an intact uterus and at least one ovary, not being pregnant or lactating, not using oral contraceptives or hormone therapy in the past three months, and having at least one menstrual cycle in the past three months. Clinic assessments began in 1996 and participants have been followed up for 15 examinations conducted approximately annually, through the most recent visit in 2015-2016. The SWAN protocols were approved by the Institutional Review Board at each site, and all participants provided written informed consent at each study visit.

Carotid ultrasound scans were performed at six sites (all sites except the Los Angeles site) at SWAN follow-up Visit 12 (2009-2011) or Visit 13 (2011-2013), with the vast majority of scans (96.6%) conducted at Visit 12. Among the 2,806 women initially enrolled at the six sites, 1,990 (70.9%) participants attended Visit 12, of whom 1,592 (80.0%) had a carotid scan at Visit 12 or Visit 13. Additionally, 14 women did not attend Visit 12, but attended and received the carotid scan at Visit 13. Thus, a total of 1,606 women had a carotid scan. From these 1,606

participants, we further excluded women who did not have all three specific measures of carotid atherosclerosis (n = 54); who did not have dietary data at both baseline and Visit 5 (n = 251); who reported too few (< 4) or too many (> 16) numbers of solid foods per day (n = 86), skipped more than 10 food items on the food frequency questionnaire (n = 2), or reported a total energy intake that was too low (< 500 kcal/d) or too high (> 5000 kcal/d) (n = 3); who self-reported having heart disease (n = 36) or stroke (n = 5) at baseline; who developed clinical heart disease (n = 29) or stroke (n = 28) during the follow-up before their carotid scans; and who had missing data for the major covariates (n = 181). After these exclusions, the final analytical sample consisted of 931 women (**Figure 2.1**).

2.3.2 Assessment of Exposures

Dietary data were collected at baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007), except at the New Jersey site, which had dietary data at baseline and Visit 5 but not at Visit 9. Diet was measured using a modification of the 1995 version of the Block food frequency questionnaire (FFQ), which has previously been validated against dietary records[88] and 24-hour recalls.[89] The FFQ included 103 food items, including 20 beverage items and 83 solid food items. Trained personnel administered the FFQ, and the participants were asked how often, on average, they consumed each item during the past year, as well as the usual portion size for each. Up to nine predefined frequencies of intake, ranging from never to ≥5 times/d, and three predefined portion sizes, ranging from small to large, were available for each beverage. Total energy intake and nutrients intake were computed by multiplying the reported frequency, the reported portion size, and the corresponding nutrient content. We computed the intakes of the

20 beverages by multiplying the reported frequency by the reported portion size. We further aggregated the beverages into eight non-overlapping groups (**Supplemental Table 2.1**), including coffee, tea, sugar-sweetened beverages (SSB), artificially sweetened beverages (ASB), fruit juices, whole milk, milk with lower fat content (2% milk, 1% milk, skim milk, and soy milk), and alcoholic beverages. The intake of each group was calculated by summing the individual items in that group. Finally, we calculated the intake for each beverage group by averaging the intakes of baseline and Visit 5. Because fewer women had dietary measures at all three visits (baseline, Visit 5, and Visit 9), the use of baseline and Visit 5 in the main analyses preserved the statistical power and allowed for the inclusion of women at the New Jersey site (including all of the Hispanic women), for whom dietary data at Visit 9 were unavailable.

2.3.3 Assessment of Outcomes

Centrally trained and certified sonographers obtained carotid ultrasound images at Visit 12 or Visit 13 using a Terason t3000 Ultrasound System (Teratech Corp, Burlington, MA) equipped with a variable frequency (5-12 MHz) linear array transducer.[90] Two digitized images were obtained for each of the left and right distal common carotid artery (CCA). From each of these four images, near and far wall intima-media thickness (IMT) measures of the CCA were obtained by electronically tracing the lumen-intima interface and the media-adventitia interface across a 1-cm segment proximal to the carotid bulb. One measurement was generated for each pixel over the area, for a total of approximately 140 measures for each segment. The average and maximal values for these measures were recorded for all four images, with the mean of the maximal readings of all four images used in the analyses. Adventitial diameter (AD) of the CCA

was measured as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interface on the far wall at end-diastole across the same CCA segments used for IMT measurement. The mean value of the average readings was used in the analyses. Sonographers at each site evaluated the presence and extent of plaque in each of five segments of the left and right carotid artery (distal and proximal CCA, carotid bulb, and proximal internal and external carotid arteries). A plaque was defined as a distinct area protruding into the vessel lumen that was at least 50% thicker than the adjacent IMT. For each segment, the degree of the plaque was graded between 0 (no observable plaque) to 3 (plaque obstructing ≥ 50% of the luminal diameter of the vessel). The grades from all segments of the combined left and right carotid artery were summed to create the plaque index.[91] The three outcomes of this study were the intima-media thickness of the common carotid artery (CCA-IMT), the adventitial diameter of the common carotid artery (CCA-AD), and the carotid plaque index. We treated CCA-IMT and CCA-AD as continuous variables and dichotomized carotid plaque index as ≥ 2 versus < 2.

2.3.4 Assessment of Covariates

Self-reported covariates at baseline included age (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), depressive symptoms (dichotomized by the Center for Epidemiologic Studies Depression Scale: ≥16 or <16),[92] menopausal status based on self-reported menstrual bleeding

patterns (dichotomized as premenopausal or early perimenopausal), smoking status (never, past, or current), non-occupational physical activity level (continuous; assessed on five-point Likert and ordinal quantitative scales with total scores ranging from 3 to 15; higher values indicate more frequent engagement in non-occupational physical activity),[93] and Alternate Healthy Eating Index (continuous; ranging from 2.5 to 87.5, with a higher value representing a better overall diet).[94, 95] Self-reported use of hormone therapy from baseline through the visit of the carotid scan was dichotomized as ever use or never use. Weight and height were measured by trained interviewers using a calibrated balance beam scale and a stadiometer, respectively, and BMI was calculated as weight in kilograms divided by squared height in meters. Blood pressure was calculated as the average of two seated measurements using a standard mercury sphygmomanometer. Blood samples were taken to measure fasting glucose, serum triglycerides, and serum high-density lipoprotein (HDL) cholesterol. Based on harmonized guidelines,[96] elevated blood pressure was defined as systolic blood pressure ≥ 130 mm Hg, or diastolic blood pressure ≥ 85 mm Hg, or antihypertensive drug treatment. Elevated fasting glucose was defined as fasting glucose ≥ 100 mg/dL or drug treatment of elevated glucose. Elevated triglycerides was defined as fasting serum triglycerides ≥ 150 mg/dL. Reduced HDL cholesterol was defined as serum HDL cholesterol < 50 mg/dL.

2.3.5 Statistical Analysis

We collapsed the average beverage intakes into prespecified categories based on prior literature and the overall distributions in the study population to detect potential nonlinear associations. Specifically, we divided coffee intake into none, > 0 to 1 serving/d, > 1 to 2

servings/d, > 2 to 4 servings/d, and > 4 servings/d. We divided tea intake into none, > 0 to 1 serving/d, > 1 to 2 servings/d, and > 2 servings/d. We divided intakes of SSB, ASB, fruit juices, milk with lower fat content, and alcoholic beverages into none, > 0 to 0.5 serving/d, > 0.5 to 1 serving/d, and > 1 serving/d. We divided whole milk intake into none, > 0 to 0.5 serving/d, and > 0.5 serving/d. We defined one serving of beverage as one medium cup (237 mL) for coffee and tea, one medium glass (237 mL) for fruit juices, whole milk, and milk with lower fat content, one medium can (355 mL) for SSB, ASB, and beer, one medium glass (148 mL) for wine, and one medium shot (44 mL) for liquor.

We estimated the associations of the categories of beverage intake with CCA-IMT and CCA-AD using linear models. CCA-IMT and CCA-AD had relatively normal distributions, and no transformations were performed. We estimated the associations of the categories of beverage intake with high carotid plaque index (carotid plaque index ≥ 2) using log-binomial models. We used modified Poisson models with robust variance estimation to achieve model convergence.[97] We used the participants who did not consume the beverage as the reference group.

The selection of confounders was based on *a priori* knowledge of risk factors for atherosclerosis and guided by the empirical exposure-covariate associations in the study population. In Model 1, we adjusted for age at the carotid scan, race/ethnicity, education level, financial strain, self-rated overall health, BMI, smoking status, non-occupational physical activity level, menopausal status, and use of hormone therapy from baseline to the visit of the carotid scan. For fruit juices and milk with lower fat content, we also adjusted for marital status; for tea, whole milk, and milk with lower fat content, we also adjusted for depressive symptoms (based on the observed exposure-covariate associations). All covariates in Model 1 were the baseline values except age and hormone therapy use. In Model 2, we additionally adjusted for

dietary covariates, including total energy intake, Alternate Healthy Eating Index (AHEI), beverage condiments (for coffee and tea only), and other beverage groups strongly correlated with each beverage. All dietary covariates in Model 2 were the average values of baseline and Visit 5. In Model 3, we additionally adjusted for physiological risk factors, including elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol, all of which were the baseline status. To test for linear trends, we assigned the median intake of each category to participants in the corresponding category as a continuous variable in the models. For beverage groups that displayed a potential nonlinear association with an outcome variable, we additionally examined the relationship non-parametrically using restricted cubic splines.[98] Tests for nonlinearity used the likelihood ratio test comparing the model with only the linear term to the model with the linear and the cubic spline terms.

We performed four sensitivity analyses to examine the robustness of the results. First, to assess selection bias due to attrition and missing data, we used inverse probability weighting to develop a non-response weight for each retained participant based on her baseline predictors of attrition (race/ethnicity, education level, financial strain, marital status, self-rated overall health, depressive symptoms, BMI, smoking status, physical activity level, menopausal status, AHEI, elevated blood pressure, elevated fasting glucose, and reduced HDL cholesterol), and repeated the analyses using the weights. Second, as some women might abstain from a beverage due to existing health conditions, we excluded participants who did not consume the beverage and used the next nonzero intake category as the reference group. Third, we restricted to the 742 women with beverage intake data from all three time points (baseline, Visit 5, and Visit 9). Fourth, we examined effect modification by race and smoking status by including a product term between

beverage intake (continuous form) and the potential effect modifier. All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC) at a two-sided alpha level of 0.05.

2.4 Results

We summarized the general characteristics of the analytical sample in **Table 2.1**. The average age of the participants at baseline was 46.4 years (SD: 2.7). Over half of the participants (54.5%) were non-Hispanic white, 24.2% were African American, 16.2% were Chinese, and 5.2% were Hispanic. At baseline, 64.2% of the women were never smokers, 25.4% were past smokers, and 10.4% were current smokers. The average BMI at baseline was 27.8 (SD: 6.9). The average CCA-IMT and CCA-AD at the visit of the carotid scan were 0.92 mm (SD: 0.14) and 7.18 mm (SD: 0.65), respectively, and 25.8% of the participants had high carotid plaque index. The stability of beverage intake over time (between baseline and Visit 5) was fair to good, with fruit juices being the least stable (Spearman $r = 0.39$) and alcoholic beverages being the most stable (Spearman $r = 0.78$). There were modest correlations between coffee and tea (Spearman $r = -0.13$), SSB and ASB (Spearman $r = -0.14$), fruit juices and whole milk (Spearman $r = 0.17$), whole milk and milk with lower fat content (Spearman $r = -0.18$), and alcoholic beverages and coffee (Spearman $r = 0.28$).

We detected an inverted J-shaped association ($P = 0.0072$) between coffee intake and CCA-IMT (**Table 2.2**). After fully adjusting for covariates, women who consumed > 0 to 1 cup/d and > 1 to 2 cups/d of coffee had a 0.031 mm (95% CI: 0.008, 0.054; $P = 0.008$) and a 0.026 mm (95% CI: 0, 0.051; $P = 0.047$) larger CCA-IMT, respectively, compared to non-drinkers. Women who consumed > 2 to 4 cups/d of coffee had comparable CCA-IMT to non-drinkers, whereas women who consumed > 4 cups/d of coffee had a 0.029 mm smaller CCA-IMT than non-drinkers, although the latter difference was not significant (95% CI: -0.073, 0.015; $P = 0.20$). Restricted cubic spline regression supported the nonlinear association between coffee

intake and CCA-IMT (*P*-curve = 0.004) (**Figure 2.2**). We did not find significant associations between coffee intake and CCA-AD or carotid plaque.

The intake of alcoholic beverages was relatively low in the study population. Sixty percent of the participants were alcohol drinkers; the mean intake among the drinkers was 0.6 serving per day and less than 4% of the participants consumed more than two servings per day. There was an inverse linear association between alcoholic beverage intake and CCA-IMT (**Table 2.3**). After adjusting for all covariates, women who consumed > 0 to 0.5 serving/d, > 0.5 to 1 serving/d, and > 1 serving/d of alcoholic beverages had a 0.009 mm ($P = 0.36$), 0.030 mm ($P = 0.05$), and 0.036 mm ($P = 0.013$) smaller CCA-IMT, respectively (P-trend = 0.009), compared to non-drinkers. When treated as a continuous variable, every one-serving per day increase in alcoholic beverage intake was associated with a 0.019 mm smaller CCA-IMT (95% CI: -0.032, -0.005; $P = 0.0077$). Restricted cubic spline regression also suggested the inverse linear association between alcoholic beverage intake and CCA-IMT (**Figure 2.3**). Women who consumed > 0 to 0.5 serving/d of alcoholic beverages had 26% lower risk of having a high carotid plaque index (RR = 0.74; 95% CI: 0.57, 0.97; $P = 0.026$), whereas consuming > 0.5 to 1 serving/d and > 1 serving/d were not significantly associated with carotid plaque. There was no significant association between alcoholic beverage intake and CCA-AD.

Women who consumed > 0 to 0.5 glass/d of whole milk had a 0.043 mm (95% CI: 0.015, 0.071; $P = 0.0025$) larger CCA-IMT compared to women who did not drink whole milk, and a 0.053 mm (95% CI: 0.012, 0.094; $P = 0.012$) larger CCA-IMT compared to women who consumed > 0.5 glass/d of whole milk (**Supplemental Table 2.2**). The difference in CCA-IMT between women who did not drink whole milk and women who drank > 0.5 glass/d was not significant. We did not find significant associations of intakes of tea, SSB, ASB, fruit juices, and

milk with lower fat content with measures of subclinical atherosclerosis (**Supplemental Tables 2.3-2.7**).

The estimates did not change appreciably after accounting for missing data using inverse probability weighting; the β coefficients for CCA-IMT and CCA-AD changed by 0.008 mm and 0.04 mm at most, respectively, and the risk ratios for carotid plaque changed by 7% at most. The results remained the same for coffee intake after excluding non-drinkers; the inverse linear association between alcoholic beverage intake and CCA-IMT was slightly less significant (*P*-trend increased from 0.0090 to 0.026 in the fully adjusted model) when non-drinkers were excluded. The key findings were qualitatively similar when restricting to the women with beverage data from all three time points, although many associations became less significant. We did not find significant multiplicative interactions between coffee intake or alcoholic beverage intake and race or smoking status (*P*-interaction > 0.10 for all models). The results from the above sensitivity analyses are not shown but are available upon request.

2.5 Discussion

This study evaluated the prospective association between beverage intake during the midlife and subclinical carotid atherosclerosis later in life, measured 14 years after baseline. We found an inverted J-shaped association between coffee intake and CCA-IMT; compared to non-drinkers, women with occasional coffee intake (≤ 2 cups/d) had a higher CCA-IMT whereas women with moderate-to-heavy intake (> 4 cups/d) had a lower CCA-IMT. We also found an inverse linear association between alcoholic beverage intake and CCA-IMT, although the study population had few women with very high alcohol intake. To our knowledge, the present study is the most comprehensive evaluation to date of the potential effect of beverage intake on subclinical carotid atherosclerosis in women.

Moderate coffee intake is associated with lower risks of coronary heart disease and stroke,[99] but the existing evidence on coffee intake and subclinical atherosclerosis is inconsistent.[40-44] In the Rotterdam Study, moderate (> 3 to 4 cups/d) and high (> 4 cups/d) coffee intakes were both associated with lower odds of severe coronary artery calcification (CAC) compared to low intake (≤ 3 cups/d).[40] Likewise, in a cross-sectional study in Korea, moderate coffee drinkers (3-5 cups/d) had a significantly lower prevalence of subclinical coronary atherosclerosis compared to non-drinkers.[42] However, no significant associations were found between coffee intake and CAC or CCA-IMT in the Coronary Artery Risk Development in Young Adults study,[41] between coffee intake and prevalent CAC in the NHLBI Family Heart Study,[44] or between regular coffee drinking (≥ 1 cup/d) and progression of CAC in the Multi-Ethnic Study of Atherosclerosis.[43] In this study, we found an inverted J-shaped association between coffee intake and CCA-IMT, with the largest CCA-IMT observed among occasional drinkers (≤ 2 cups/d) and the smallest CCA-IMT among moderate-to-heavy drinkers (> 4

cups/d). Antioxidant and anti-inflammatory phenolic compounds in coffee, such as chlorogenic acid, can improve insulin sensitivity and β-cell function,[35] prevent oxidation of LDL cholesterol,[34] lessen endothelial dysfunction,[33] and reduce blood pressure,[36] all of which are important components of the atherosclerotic process. It also has been hypothesized that phytoestrogens such as lignans from coffee may partially replace estrogen after menopause and protect against atherosclerosis.[31] On the other hand, caffeine[30] and chlorogenic acid[32] may elevate the plasma homocysteine level; diterpenoid molecules in unfiltered coffee such as cafestol and kahweol can increase serum total cholesterol levels.[100] The present study suggests that the beneficial properties of the coffee constituents are more likely to outweigh the harmful effects among moderate-to-heavy drinkers (> 4 cups/d) compared to among occasional drinkers (≤ 2 cups/d), which is in line with previous evidence on clinical CVD showing that daily intake of 3 to 5 cups of coffee confers the maximal cardiovascular benefits.[99]

There is a relatively well-documented J-shaped association between alcohol intake and the incidence of clinical CVD, with the lowest risk observed for people who consumed one serving of alcoholic beverages per day.[85] Prior studies on the effect of alcohol intake on subclinical atherosclerosis have conflicting results, with some studies reporting a J-shaped association in both women and men,[49-51] some finding an association in men but not in women,[52, 53] and some failing to detect a meaningful association at all.[54-56] We found that alcoholic beverage intake was inversely and linearly associated with CCA-IMT. This finding does not contradict previous studies that found a J-shaped association with potential harmful effects of alcohol at excessive intake,[49-51] because the intake of alcoholic beverages in this study population was fairly low with less than 4% of the women consuming more than two servings per day. It is likely that alcoholic beverages are protective against atherosclerosis when consumed in

moderation but will have no effect or even a detrimental effect on atherosclerosis when heavily consumed. HDL cholesterol and hypertension have been proposed as potential mediators of the effect of alcohol intake on atherosclerosis.[50, 52] In the present study, the associations observed for alcohol intake persisted even after adjusting for HDL cholesterol and hypertension, suggesting the possibility of alternative mechanisms, such as changes in inflammatory and hemostatic factors.[101]

Polyphenols in tea, most notably flavonoids, have been shown in cellular and animal studies to have antioxidant, antithrombotic, and anti-inflammatory properties that reduce oxidation of LDL cholesterol,[37] inhibit platelet aggregation,[39] and slower the development of fatty streaks.[38] Epidemiologic studies suggest that tea intake may be associated with less atherosclerosis of the aorta and coronary arteries, but the evidence for carotid atherosclerosis is inconsistent.[41, 43, 45, 46] As the protective effect of tea intake is likely stronger with high intake, the discrepant results in the literature may also be partially attributable to the lower level of tea intake in the U.S. compared to in European countries. For example, the average daily tea intake was approximately 1 cup (237 mL) in this study compared to 1.9 cups (445 mL) in the Rotterdam Study.[45] Approximately five percent of the women in the present study consumed ≥ 3 cups/d of tea compared to 12.8% in the French Three-City Study.[46]

Overwhelming evidence suggests that the intake of energy-dense beverages can lead to weight gain and the development of insulin resistance, inflammation, hypertension, visceral adiposity, and atherogenic dyslipidemia.[29] The potential effects of ASB and fruit juices on atherosclerosis are less studied. The lack of a clear association for SSB from this study is somewhat surprising and may in part due to reverse causation as women who were aware of higher cardiovascular risk might intentionally reduce their SSB intake. The effect of ASB on

cardiometabolic health warrants further investigations as ASB may be a reasonable replacement to reduce the intake of SSB.[84] Whole milk intake has long been suspected to be a CVD risk factor in adults due to its high saturated fat content and excess calories. Limited prior studies did not find a significant association between milk intake and subclinical atherosclerosis.[47, 48] We found that consuming $\leq$ 0.5 glass/d of whole milk was associated with larger CCA-IMT than consuming none and consuming > 0.5 glass/d. Future work is needed to confirm this finding as only a small proportion of women in our study population were whole milk drinkers.

The three outcomes in this study represent physiologically different aspects of the atherosclerotic processes. CCA-IMT is the early marker of atherosclerosis and reflects the thickening of the vessel wall due to lipid deposition, chronic inflammation, and infiltration of immunological cells;[10] increase in CCA-IMT may also be in response to hemodynamic changes such as elevated blood pressure.[102] The development of a plaque is the hallmark of atherosclerosis that results from the prolonged accumulation of inflammation, lipid deposition, and calcification.[10] The accumulation of fatty deposits that leads to a thickening of CCA-IMT precedes plaque formation, which may explain the presence of a dose-response relationship of alcoholic beverages with CCA-IMT but not with carotid plaque. An elevated CCA-AD represents dilation of the vessel, disturbance in blood flow, and potentially less flexibility to dilate further in response to stimuli. CCA-AD has been shown as a marker of vascular remodeling.[11] The overall lack of observed associations for CCA-AD in this study suggests that beverage intake may not be associated with the vascular remodeling aspect of atherosclerosis.

The primary strengths of this study include the focus on midlife women from diverse racial/ethnic backgrounds and the use of repeated measures of beverage intake. This study also has some potential limitations. First, carotid atherosclerosis was measured only once. Without

the baseline measures, we were unable to evaluate the change of subclinical atherosclerosis over time. Second, the self-reported beverage intake inevitably had some measurement error, which was reduced by using repeated measures from a validated FFQ and by excluding participants with low-quality FFQ data. Third, we could not separately examine the effects of extremely high intakes for some beverages (e.g., > 3 cups/d of tea or > 2 servings/d of SSB) because such extreme values were rare in the analytical sample. Fourth, we had no information on coffee brewing methods or decaffeinated coffee intake. Fifth, although we extensively adjusted for covariates, it was not possible to eliminate residual confounding. For example, the estimates may be confounded by other dietary factors. However, we used the AHEI, a well-established dietary index that incorporates a wide range of important foods and nutrients and the types of protein and fats,[94, 95] to adjust for the overall diet. Some other potential unmeasured confounders include occupation status, existing health conditions, and underlying health concerns that influenced the participants' beverage intakes. Therefore, it was not possible to draw any conclusions on causal effects from this study. Last but not least, due to the large number of models examined, this study was subject to multiple comparisons. After the false discovery rate adjustment[103] for the multiple testing of the 24 beverage-outcome combinations, none of the adjusted *P* values remained significant. However, all the primary analyses were based on *a priori* hypotheses and have been reported either in the main text or the supplementary tables. Nevertheless, some of the significant associations might be due to random error, and the results should be interpreted with caution. Future studies are certainly needed to confirm the findings.

In conclusion, this prospective study indicates that occasional coffee intake during the midlife is associated with more subclinical carotid atherosclerosis later in life whereas moderate-to-heavy intake may be associated with less subclinical carotid atherosclerosis. This study also

suggests that moderate intake of alcoholic beverages during the midlife is associated with less atherosclerosis. Future work should focus on the determination of the optimal beverage intake profile for maximum cardiovascular benefits in women undergoing the menopausal transition.

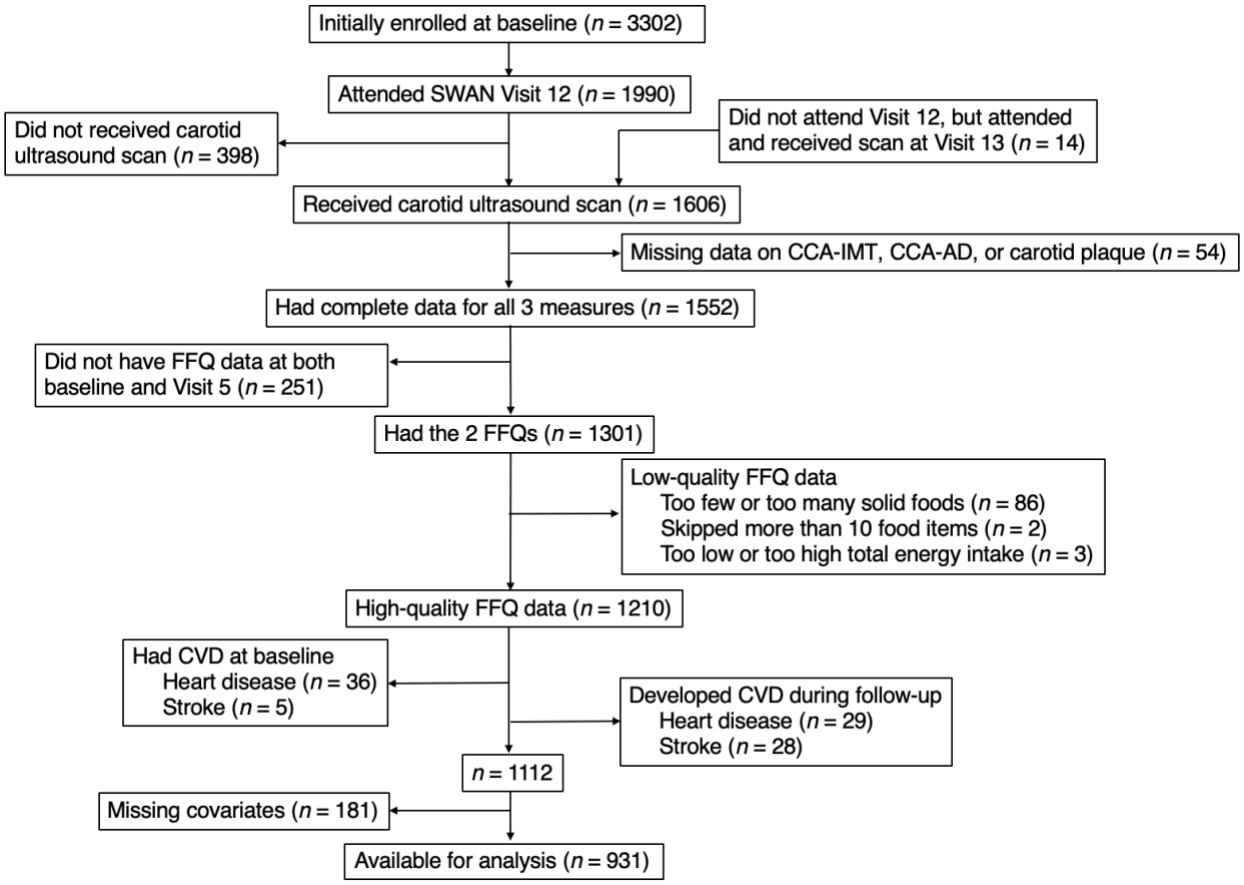

Figure 2.1 Exclusion flow of participants for the association between beverage intake and subclinical carotid atherosclerosis in the Study of Women's Health Across the Nation. Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CVD; cardiovascular disease; FFQ, food frequency questionnaire; IMT, intima-media thickness.

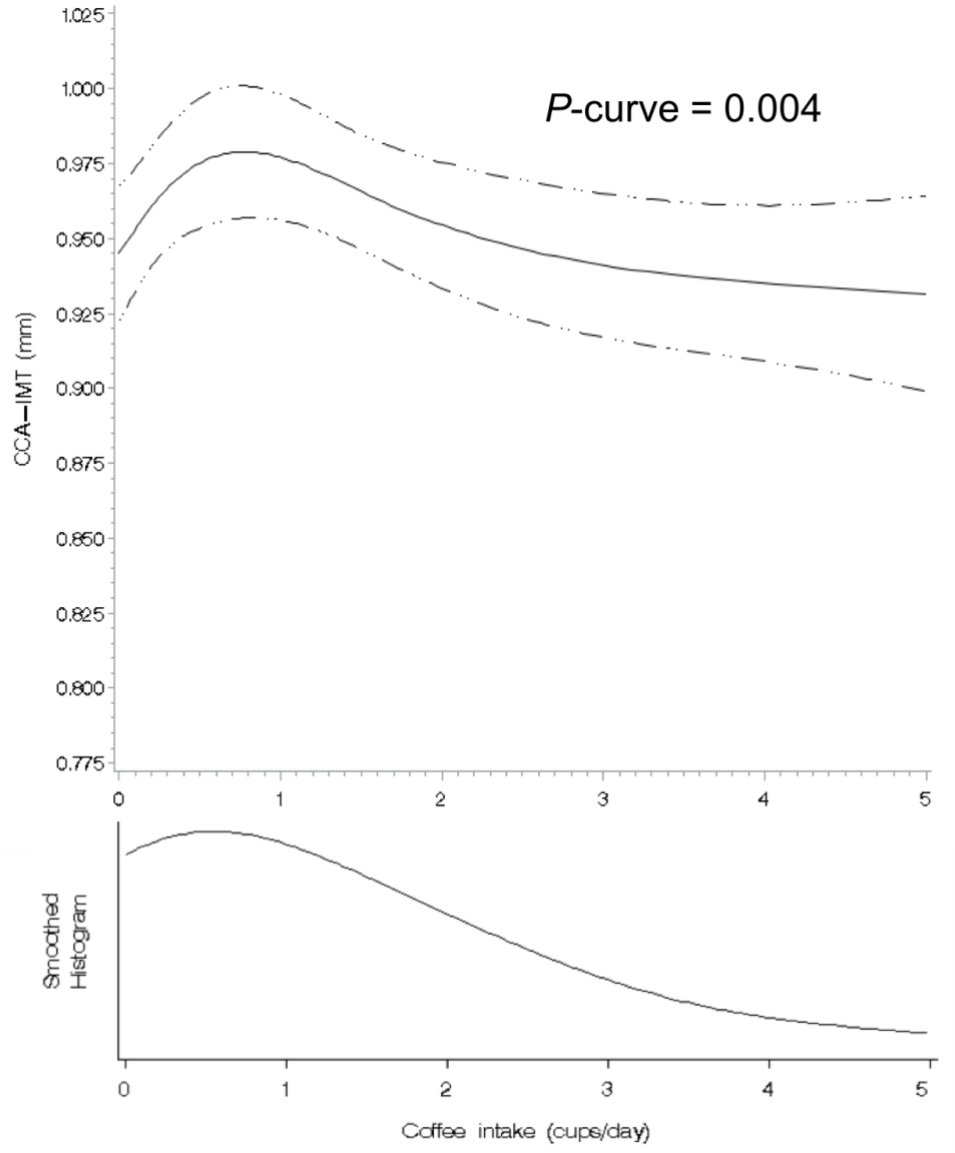

Figure 2.2 Association between coffee intake and common carotid artery intima-media thickness using restricted cubic splines. The solid line represents the predicted least squares means computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). The dashed lines represent the 95% confidence limits. Four knots were placed at 5th, 35th, 65th, and 95th percentiles of the sample distribution corresponding to 0, 0.24, 1.52, and 4.50 cups/d, respectively. One serving of coffee was defined as one medium cup (237 mL). *P*-curve was computed using the likelihood ratio test comparing the model with only the linear term to the model with the linear and the cubic spline terms. The model was adjusted for age at the carotid scan, race/ethnicity, education level, financial strain, self-rated overall health, BMI, smoking status, non-occupational physical activity level, menopausal status, use of hormone therapy from baseline to the visit of the carotid scan, total energy intake, Alternate Healthy Eating Index, intake of tea, intake of beverage condiments, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol. Extreme coffee intake (> 5 cups/d) was trimmed from the graph due to sparse data. Abbreviations: CCA, common carotid artery; IMT, intima-media thickness.

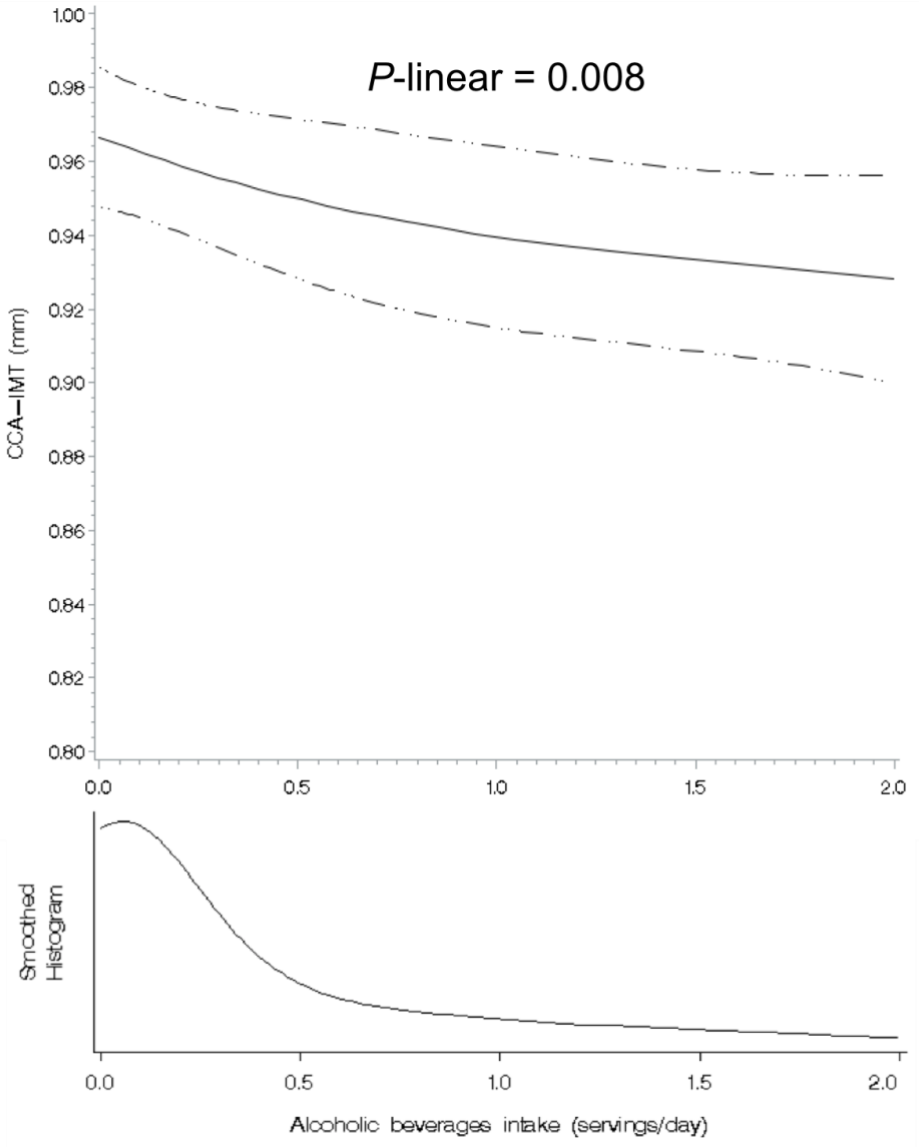

Figure 2.3 Association between alcoholic beverage intake and common carotid artery intima-media thickness using restricted cubic splines. The solid line represents the predicted least squares means computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). The dashed lines represent the 95% confidence limits. Three knots were placed at 5th, 50th, and 95th percentiles of the sample distribution, corresponding to 0, 0.07, and 1.74 servings/d, respectively. One serving of alcoholic beverages was defined as one medium can (355 mL) for beer, one medium glass (148 mL) for wine, and one medium shot (44 mL) for liquor. The model was adjusted for age at the carotid scan, race/ethnicity, education level, financial strain, self-rated overall health, BMI, smoking status, non-occupational physical activity level, menopausal status, use of hormone therapy from baseline to the visit of the carotid scan, total energy intake, Alternate Healthy Eating Index (excluding the alcohol component), coffee intake, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol. Extreme alcohol intake (> 2 servings/d) was trimmed from the graph due to sparse data. Abbreviations: CCA, common carotid artery; IMT, intima-media thickness.

Table 2.1 General characteristics and beverage intake among 931 participants of the Study of Women's Health Across the Nation[a]

	n = 931
Major covariates	
Age at baseline, year, mean (SD)	46.4 (2.7)
Age at the carotid scan, year, mean (SD)	60.3 (2.7)
Race and ethnicity	
- African American, %	24.2
- Hispanic, %	5.2
- Chinese, %	16.2
- Non-Hispanic white, %	54.5
Education level	
- High school or less, %	20.3
- Some college, %	29.8
- College degree/post-college, %	50.0
Somewhat/very hard to pay for basics, %	30.3
Marital status	
- Single/never married, %	13.1
- Married/living as if married, %	70.1
- Separated/widowed/divorced, %	16.8
Self-rated overall health	
- Excellent/very good, %	63.9
- Good, %	27.4
- Fair/poor, %	8.7
Depressive symptoms[b], %	21.6
Total energy intake[c], kcal/d, mean (SD)	1784.0 (552.0)
BMI, mean (SD)	27.8 (6.9)
Smoking status	
- Never, %	64.2
- Past, %	25.4
- Current, %	10.4
Non-occupational physical activity[d], mean (SD)	7.8 (1.8)
Alternate Healthy Eating Index[c, e], mean (SD)	37.4 (9.1)
Menopausal status	
- Early perimenopausal, %	42.9
- Premenopausal, %	57.1
Hormone therapy use[f], %	42.8
Elevated blood pressure, %	25.6
Elevated fasting glucose, %	19.6
Elevated triglycerides, %	16.5
Reduced HDL cholesterol, %	31.0
Beverage intake[g]	
Coffee drinkers, %	74.2
- Intake of coffee, cups/d, mean (SD)	1.7 (1.5)
Tea drinkers, %	74.1
- Intake of tea, cups/d, mean (SD)	0.9 (1.2)
Sugar-sweetened beverages drinkers, %	79.6
- Intake of SSB, cans/d, mean (SD)	0.7 (0.8)
Artificially sweetened beverages drinkers, %	50.6
- Intake of ASB, cans/d, mean (SD)	0.7 (0.9)
Fruit juices drinkers, %	85.8
- Intake of fruit juices, glasses/d, mean (SD)	0.6 (0.6)
Whole milk drinkers, %	17.5
- Intake of whole milk, glasses/d, mean (SD)	0.6 (0.9)
Milk with lower fat content drinkers, %	89.9
- Intake of milk with lower fat content, glasses/d, mean (SD)	0.9 (0.9)
Alcoholic beverages drinkers, %	60.0
- Intake of alcoholic beverages, servings/d, mean (SD)	0.6 (0.7)
Subclinical atherosclerosis	

CCA-IMT[h], mm, mean (SD)	0.9 (0.1)
CCA-AD[h], mm, mean (SD)	7.2 (0.7)
High carotid plaque index[h, i], %	25.8

Abbreviations: AD, adventitial diameter; ASB, artificially sweetened beverages; CCA, common carotid artery; IMT, intima-media thickness; SD, standard deviation; SSB, sugar-sweetened beverages.

[a] Values are means (standard deviations) for continuous variables and percentages for categorical variables. Values of polytomous variables may not sum to 100% due to rounding. The variables are the baseline measures unless specified otherwise.

[b] Defined as the Center for Epidemiologic Studies Depression Scale ≥ 16.

[c] Average value of baseline and Visit 5.

[d] Assessed on five-point Likert and ordinal quantitative scales with total scores ranging from 3 to 15, with higher values indicating more frequent engagement in non-occupational physical activity.

[e] Computed by assessing nine dietary components including vegetables, fruit, nuts and legumes, the ratio of white to red meat, cereal fiber, *trans* fat, the ratio of PUFA to SFA, multivitamin use, and alcohol intake. Each component contributes 0-10 points to the total score, except the dichotomous multivitamin intake component, which contributes either 2.5 points for no long-term use or 7.5 points for long-term use. A maximum score indicates that the recommendation for that component was fully met, whereas a minimum score represents the least healthy dietary behavior for that component. Intermediate intakes were scored proportionally between the minimum score and the maximum score. The scores of the nine components were summed to obtain the total score ranging from 2.5 (worst overall diet) to 87.5 (best overall diet).

[f] Defined as reported use of hormone therapy at any time from baseline to the visit of the carotid scan.

[g] Values are the averages of baseline and Visit 5. One serving was defined as one medium cup (237 mL) for coffee and tea, one medium glass (237 mL) for fruit juices, whole milk, and milk with lower fat content, one medium can (355 mL) for sugar-sweetened beverages, artificially sweetened beverages, and beer, one medium glass (148 mL) for wine, and one medium shot (44 mL) for liquor. The intakes were calculated among the drinkers only.

[h] Measured either at Visit 12 (2009-2011) or Visit 13 (2011-2013).

[i] Defined as carotid plaque index ≥ 2.

Table 2.2 Average coffee intake and subclinical carotid atherosclerosis among 931 participants of the Study of Women's Health Across the Nation[a]

Coffee, servings/d						*P*	*P*-trend[b]
	None	> 0 to 1	> 1 to 2	> 2 to 4	> 4		
n (%)	240 (25.78)	261 (28.03)	199 (21.37)	177 (19.01)	54 (5.80)		
CCA-IMT, mm							
Model 1[c]	0.931 (0.909, 0.953)	0.960 (0.940, 0.980)[f]	0.953 (0.932, 0.974)	0.936 (0.913, 0.958)	0.903 (0.866, 0.940)	0.013	0.13
Model 2[d]	0.930 (0.908, 0.953)	0.960 (0.939, 0.980)[f]	0.954 (0.933, 0.975)	0.937 (0.914, 0.960)	0.900 (0.860, 0.939)	0.0098	0.15
Model 3[e]	0.942 (0.918, 0.966)	0.973 (0.950, 0.995)[g]	0.967 (0.944, 0.991)[f]	0.951 (0.926, 0.976)	0.913 (0.872, 0.954)	0.0072	0.18
CCA-AD, mm							
Model 1[c]	7.26 (7.16, 7.37)	7.28 (7.19, 7.38)	7.27 (7.17, 7.37)	7.29 (7.18, 7.39)	7.12 (6.94, 7.29)	0.50	0.30
Model 2[d]	7.25 (7.15, 7.36)	7.28 (7.19, 7.38)	7.27 (7.17, 7.37)	7.30 (7.19, 7.41)	7.13 (6.94, 7.32)	0.53	0.52
Model 3[e]	7.31 (7.20, 7.43)	7.35 (7.24, 7.45)	7.34 (7.23, 7.45)	7.36 (7.24, 7.48)	7.21 (7.02, 7.41)	0.61	0.64
Carotid plaque							
Model 1[c]	*Ref*	1.09 (0.80, 1.49)	1.19 (0.85, 1.67)	1.27 (0.91, 1.78)	1.26 (0.81, 1.95)	0.68	0.18
Model 2[d]	*Ref*	1.06 (0.78, 1.45)	1.14 (0.81, 1.61)	1.19 (0.84, 1.68)	1.05 (0.63, 1.75)	0.86	0.60
Model 3[e]	*Ref*	1.08 (0.79, 1.47)	1.18 (0.84, 1.66)	1.26 (0.89, 1.78)	1.03 (0.61, 1.76)	0.66	0.56

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; IMT, intima-media thickness.

[a] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to handle model convergence issues. One serving of coffee was defined as one medium cup (237 mL).

[b] Computed by assigning the median intake of each category to participants in the corresponding category as a continuous variable.

[c] Adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), BMI (continuous), smoking status (never, past, or current), non-occupational physical activity level (continuous), menopausal status (premenopausal or early perimenopausal), and use of hormone therapy from baseline to the visit of the carotid scan (ever or never). The baseline covariates were used unless specified otherwise.

[d] Model 1 + dietary covariates: The dietary covariates included total energy intake, Alternate Healthy Eating Index, intake of tea, and intake of beverage condiments, all of which were continuous and the average values of baseline and Visit 5.

[e] Model 2 + physiological risk factors: The physiological risk factors included elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol, all binary and measured at baseline.

[f] $P<0.05$ (compared to the reference group).

[g] $P<0.01$ (compared to the reference group).

Table 2.3 Average intake of alcoholic beverages and subclinical carotid atherosclerosis among 931 participants of the Study of Women's Health Across the Nation[a]

Alcoholic beverages, servings/d					*P*	*P*-trend[b]
	None	> 0 to 0.5	> 0.5 to 1	> 1		
n (%)	372 (39.96)	340 (36.52)	98 (10.53)	121 (13.00)		
CCA-IMT, mm						
Model 1[c]	0.955 (0.937, 0.973)	0.945 (0.926, 0.964)	0.925 (0.897, 0.953)[f]	0.920 (0.893, 0.947)[f]	0.046	0.0095
Model 2[d]	0.955 (0.937, 0.974)	0.946 (0.927, 0.965)	0.927 (0.898, 0.955)	0.921 (0.894, 0.949)[f]	0.064	0.013
Model 3[e]	0.968 (0.948, 0.989)	0.959 (0.938, 0.980)	0.939 (0.909, 0.969)	0.932 (0.903, 0.961)[f]	0.048	0.0090
CCA-AD, mm						
Model 1[c]	7.29 (7.20, 7.38)	7.26 (7.17, 7.35)	7.21 (7.08, 7.34)	7.24 (7.11, 7.37)	0.70	0.43
Model 2[d]	7.29 (7.20, 7.38)	7.26 (7.17, 7.35)	7.22 (7.08, 7.35)	7.25 (7.13, 7.38)	0.77	0.59
Model 3[e]	7.35 (7.26, 7.45)	7.33 (7.23, 7.43)	7.28 (7.14, 7.42)	7.30 (7.17, 7.44)	0.74	0.43
Carotid plaque						
Model 1[c]	*Ref*	0.74 (0.57, 0.96)[f]	1.00 (0.69, 1.46)	1.13 (0.82, 1.57)	0.034	0.11
Model 2[d]	*Ref*	0.73 (0.56, 0.95)[f]	1.00 (0.69, 1.46)	1.09 (0.79, 1.52)	0.037	0.16
Model 3[e]	*Ref*	0.74 (0.57, 0.97)[f]	1.04 (0.71, 1.52)	1.11 (0.80, 1.53)	0.039	0.13

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; IMT, intima-media thickness.

[a] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to handle model convergence issues. One serving of alcoholic beverages was defined as one medium can (355 mL) for beer, one medium glass (148 mL) for wine, and one medium shot (44 mL) for liquor.

[b] Computed by assigning the median intake of each category to participants in the corresponding category as a continuous variable.

[c] Adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), BMI (continuous), smoking status (never, past, or current), non-occupational physical activity level (continuous), menopausal status (premenopausal or early perimenopausal), and use of hormone therapy from baseline to the visit of the carotid scan (ever or never). The baseline covariates were used unless specified otherwise.

[d] Model 1 + dietary covariates: The dietary covariates included total energy intake, Alternate Healthy Eating Index (excluding the alcohol component), and intake of coffee, all of which were continuous and the average values of baseline and Visit 5.

[e] Model 2 + physiological risk factors: The physiological risk factors included elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol, all binary and measured at baseline.

[f] $P<0.05$ (compared to the reference group).

Supplemental Table 2.1 Beverage items and beverage groups in the Study of Women's Health Across the Nation

Beverage item	Beverage group
Caffeinated coffee	Coffee
Green tea	Tea
Black tea, English tea, or Chinese tea	
Chinese herbs soup or tea	
Kool-Aid, Hi-C, or other drinks with added vitamin C	Sugar-sweetened beverages
Snapple, Calistoga, sweetened bottled waters or iced teas	
Regular cola soft drinks	
Diet cola soft drinks	Artificially sweetened beverages
Orange juice or grapefruit juice	Fruit juices
Apple juice or grape juice	
Whole milk or whole chocolate milk	Whole milk
2% milk or 2% chocolate milk	Milk with lower fat content
Skim milk or 1% milk	
Soy milk	
Beer	Alcoholic beverages
Wine or wine coolers	
Liquor or mixed drinks	
Cream, half and half, or nondairy creamer in coffee or tea	Beverage condiments
Milk in coffee or tea	
Sugar or honey in coffee or tea or on cereal	

Supplemental Table 2.2 Average whole milk intake and subclinical carotid atherosclerosis among 931 participants of the Study of Women's Health Across the Nation[a]

Whole milk, servings/d				*P*	*P*-trend[b]
	None	> 0 to 0.5	> 0.5		
n (%)	768 (82.49)	96 (10.31)	67 (7.20)		
CCA-IMT, mm					
Model 1[c]	0.937 (0.920, 0.954)	0.983 (0.955, 1.011)[f]	0.931 (0.898, 0.964)	0.0033	0.72
Model 2[d]	0.937 (0.920, 0.954)	0.982 (0.954, 1.010)[f]	0.929 (0.895, 0.962)	0.0038	0.63
Model 3[e]	0.949 (0.930, 0.969)	0.992 (0.963, 1.022)[f]	0.940 (0.905, 0.974)	0.0056	0.59
CCA-AD, mm					
Model 1[c]	7.26 (7.18, 7.34)	7.31 (7.18, 7.44)	7.24 (7.08, 7.40)	0.72	0.79
Model 2[d]	7.26 (7.18, 7.34)	7.31 (7.17, 7.44)	7.25 (7.09, 7.41)	0.76	0.88
Model 3[e]	7.32 (7.23, 7.41)	7.36 (7.22, 7.50)	7.31 (7.15, 7.48)	0.84	0.90
Carotid plaque					
Model 1[c]	*Ref*	1.26 (0.92, 1.71)	1.21 (0.80, 1.83)	0.32	0.36
Model 2[d]	*Ref*	1.27 (0.93, 1.73)	1.16 (0.75, 1.79)	0.35	0.49
Model 3[e]	*Ref*	1.21 (0.89, 1.63)	1.12 (0.73, 1.74)	0.48	0.59

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; IMT, intima-media thickness.

[a] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to handle model convergence issues. One serving of whole milk was defined as one medium glass (237 mL).

[b] Computed by assigning the median intake of each category to participants in the corresponding category as a continuous variable.

[c] Adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), BMI (continuous), smoking status (never, past, or current), non-occupational physical activity level (continuous), menopausal status (premenopausal or early perimenopausal), and use of hormone therapy from baseline to the visit of the carotid scan (ever or never). The baseline covariates were used unless specified otherwise.

[d] Model 1 + dietary covariates: The dietary covariates included total energy intake, Alternate Healthy Eating Index, and intake of milk with lower fat content, all of which were continuous and the average values of baseline and Visit 5.

[e] Model 2 + physiological risk factors: The physiological risk factors included elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol, all binary and measured at baseline.

[f] $P<0.01$ (compared to the reference group).

Supplemental Table 2.3 Average tea intake and subclinical carotid atherosclerosis among 931 participants of the Study of Women's Health Across the Nation[a]

Tea, servings/d					*P*	*P*-trend[b]
	None	> 0 to 1	> 1 to 2	> 2		
n (%)	241 (25.89)	488 (52.42)	107 (11.49)	95 (10.20)		
CCA-IMT, mm						
Model 1[c]	0.945 (0.925, 0.965)	0.939 (0.921, 0.957)	0.942 (0.913, 0.970)	0.952 (0.922, 0.983)	0.77	0.47
Model 2[d]	0.945 (0.925, 0.965)	0.940 (0.922, 0.958)	0.944 (0.915, 0.972)	0.954 (0.924, 0.985)	0.80	0.43
Model 3[e]	0.957 (0.935, 0.979)	0.952 (0.932, 0.972)	0.956 (0.926, 0.986)	0.969 (0.936, 1.001)	0.73	0.35
CCA-AD, mm						
Model 1[c]	7.30 (7.20, 7.39)	7.23 (7.15, 7.32)	7.33 (7.19, 7.46)	7.24 (7.10, 7.39)	0.32	0.89
Model 2[d]	7.30 (7.20, 7.39)	7.23 (7.15, 7.32)	7.34 (7.20, 7.47)	7.26 (7.12, 7.41)	0.33	0.68
Model 3[e]	7.36 (7.26, 7.46)	7.29 (7.20, 7.39)	7.40 (7.26, 7.54)	7.33 (7.18, 7.49)	0.28	0.58
Carotid plaque						
Model 1[c]	*Ref*	0.99 (0.76, 1.29)	1.16 (0.80, 1.67)	1.11 (0.74, 1.65)	0.80	0.43
Model 2[d]	*Ref*	0.99 (0.76, 1.29)	1.14 (0.79, 1.65)	1.02 (0.68, 1.52)	0.86	0.74
Model 3[e]	*Ref*	1.00 (0.77, 1.30)	1.14 (0.80, 1.64)	1.07 (0.71, 1.61)	0.88	0.60

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; IMT, intima-media thickness.

[a] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to handle model convergence issues. One serving of tea was defined as one medium cup (237 mL).

[b] Computed by assigning the median intake of each category to participants in the corresponding category as a continuous variable.

[c] Adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), BMI (continuous), smoking status (never, past, or current), non-occupational physical activity level (continuous), menopausal status (premenopausal or early perimenopausal), and use of hormone therapy from baseline to the visit of the carotid scan (ever or never). The baseline covariates were used unless specified otherwise.

[d] Model 1 + dietary covariates: The dietary covariates included total energy intake, Alternate Healthy Eating Index, intake of coffee, and intake of beverage condiments, all of which were continuous and the average values of baseline and Visit 5

[e] Model 2 + physiological risk factors: The physiological risk factors included elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol, all binary and measured at baseline.

Supplemental Table 2.4 Average sugar-sweetened beverages intake and subclinical carotid atherosclerosis among 931 participants of the Study of Women's Health Across the Nation[a]

SSB, servings/d					*P*	*P*-trend[b]
	None	> 0 to 0.5	> 0.5 to 1	> 1		
n (%)	190 (20.41)	448 (48.12)	117 (12.57)	176 (18.90)		
CCA-IMT, mm						
Model 1[c]	0.944 (0.921, 0.966)	0.943 (0.926, 0.960)	0.937 (0.911, 0.964)	0.951 (0.928, 0.974)	0.84	0.56
Model 2[d]	0.944 (0.921, 0.966)	0.944 (0.927, 0.962)	0.939 (0.912, 0.965)	0.953 (0.929, 0.976)	0.81	0.51
Model 3[e]	0.956 (0.932, 0.981)	0.956 (0.936, 0.976)	0.948 (0.920, 0.976)	0.966 (0.940, 0.992)	0.71	0.51
CCA-AD, mm						
Model 1[c]	7.30 (7.19, 7.40)	7.26 (7.18, 7.34)	7.26 (7.13, 7.39)	7.25 (7.14, 7.36)	0.89	0.66
Model 2[d]	7.30 (7.19, 7.40)	7.26 (7.18, 7.35)	7.26 (7.14, 7.39)	7.26 (7.14, 7.37)	0.92	0.72
Model 3[e]	7.36 (7.24, 7.48)	7.32 (7.23, 7.41)	7.31 (7.18, 7.45)	7.34 (7.21, 7.46)	0.88	0.98
Carotid plaque						
Model 1[c]	*Ref*	1.21 (0.90, 1.62)	1.22 (0.82, 1.83)	1.27 (0.89, 1.80)	0.50	0.42
Model 2[d]	*Ref*	1.28 (0.96, 1.71)	1.36 (0.91, 2.05)	1.43 (1.00, 2.03)[f]	0.18	0.17
Model 3[e]	*Ref*	1.26 (0.94, 1.70)	1.25 (0.84, 1.88)	1.29 (0.90, 1.86)	0.38	0.49

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; IMT, intima-media thickness; SSB, sugar-sweetened beverages.

[a] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to handle model convergence issues. One serving of SSB was defined as one medium can (355 mL).

[b] Computed by assigning the median intake of each category to participants in the corresponding category as a continuous variable.

[c] Adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), BMI (continuous), smoking status (never, past, or current), non-occupational physical activity level (continuous), menopausal status (premenopausal or early perimenopausal), and use of hormone therapy from baseline to the visit of the carotid scan (ever or never). The baseline covariates were used unless specified otherwise.

[d] Model 1 + dietary covariates: The dietary covariates included Alternate Healthy Eating Index and intake of artificially sweetened beverages, both of which were continuous and the average values of baseline and Visit 5.

[e] Model 2 + physiological risk factors: The physiological risk factors included total energy intake, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol. Total energy intake was the continuous average values of baseline and Visit 5, and the other four were all binary and measured at baseline.

[f] $P<0.05$ (compared to the reference group).

Supplemental Table 2.5 Average artificially sweetened beverages intake and subclinical carotid atherosclerosis among 931 participants of the Study of Women's Health Across the Nation[a]

ASB, servings/d					*P*	*P*-trend[b]
	None	> 0 to 0.5	> 0.5 to 1	> 1		
n (%)	460 (49.41)	281 (30.18)	94 (10.10)	96 (10.31)		
CCA-IMT, mm						
Model 1[c]	0.942 (0.925, 0.960)	0.938 (0.918, 0.957)	0.968 (0.939, 0.998)	0.956 (0.926, 0.986)	0.19	0.18
Model 2[d]	0.942 (0.925, 0.959)	0.939 (0.919, 0.959)	0.968 (0.938, 0.998)	0.956 (0.926, 0.987)	0.22	0.17
Model 3[e]	0.955 (0.935, 0.974)	0.951 (0.929, 0.973)	0.979 (0.948, 1.011)	0.967 (0.936, 0.998)	0.27	0.22
CCA-AD, mm						
Model 1[c]	7.26 (7.17, 7.34)	7.23 (7.14, 7.33)	7.38 (7.24, 7.52)	7.34 (7.20, 7.48)	0.15	0.10
Model 2[d]	7.25 (7.17, 7.34)	7.24 (7.14, 7.33)	7.38 (7.24, 7.52)	7.34 (7.20, 7.48)	0.18	0.11
Model 3[e]	7.32 (7.23, 7.41)	7.30 (7.19, 7.40)	7.44 (7.30, 7.59)	7.40 (7.25, 7.54)	0.16	0.12
Carotid plaque						
Model 1[c]	*Ref*	1.22 (0.94, 1.59)	1.49 (1.06, 2.09)[f]	1.36 (0.98, 1.91)	0.092	0.070
Model 2[d]	*Ref*	1.22 (0.94, 1.59)	1.51 (1.07, 2.11)[f]	1.40 (0.99, 1.98)	0.081	0.056
Model 3[e]	*Ref*	1.24 (0.96, 1.62)	1.47 (1.04, 2.06)[f]	1.32 (0.94, 1.85)	0.11	0.13

Abbreviations: AD, adventitial diameter; ASB, artificially sweetened beverages; CCA, common carotid artery; IMT, intima-media thickness.

[a] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to handle model convergence issues. One serving of artificially sweetened beverages was defined as one medium can (355 mL).

[b] Computed by assigning the median intake of each category to participants in the corresponding category as a continuous variable.

[c] Adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), BMI (continuous), smoking status (never, past, or current), non-occupational physical activity level (continuous), menopausal status (premenopausal or early perimenopausal), and use of hormone therapy from baseline to the visit of the carotid scan (ever or never). The baseline covariates were used unless specified otherwise.

[d] Model 1 + dietary covariates: The dietary covariates included Alternate Healthy Eating Index and intake of sugar-sweetened beverages, both of which were continuous and the average values of baseline and Visit 5.

[e] Model 2 + physiological risk factors: The physiological risk factors included total energy intake, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol. Total energy intake was the continuous average values of baseline and Visit 5, and the other four were all binary and measured at baseline.

[f] $P<0.05$ (compared to the reference group).

Supplemental Table 2.6 Average fruit juices intake and subclinical carotid atherosclerosis among 931 participants of the Study of Women's Health Across the Nation[a]

Fruit juices, servings/d					*P*	*P*-trend[b]
	None	> 0 to 0.5	> 0.5 to 1	> 1		
n (%)	132 (14.18)	474 (50.91)	195 (20.95)	130 (13.96)		
CCA-IMT, mm						
Model 1[c]	0.957 (0.930, 0.984)	0.941 (0.922, 0.960)	0.950 (0.927, 0.973)	0.953 (0.927, 0.978)	0.55	0.58
Model 2[d]	0.956 (0.929, 0.983)	0.940 (0.922, 0.959)	0.952 (0.928, 0.975)	0.956 (0.930, 0.983)	0.44	0.38
Model 3[e]	0.969 (0.940, 0.997)	0.954 (0.932, 0.975)	0.964 (0.939, 0.989)	0.965 (0.938, 0.993)	0.55	0.57
CCA-AD, mm						
Model 1[c]	7.28 (7.15, 7.41)	7.28 (7.19, 7.37)	7.25 (7.14, 7.36)	7.25 (7.13, 7.37)	0.91	0.51
Model 2[d]	7.27 (7.15, 7.40)	7.27 (7.18, 7.36)	7.25 (7.14, 7.36)	7.26 (7.13, 7.38)	0.99	0.80
Model 3[e]	7.33 (7.20, 7.47)	7.34 (7.24, 7.44)	7.32 (7.20, 7.44)	7.31 (7.18, 7.44)	0.93	0.56
Carotid plaque						
Model 1[c]	*Ref*	1.12 (0.83, 1.51)	1.15 (0.81, 1.64)	1.01 (0.66, 1.53)	0.79	0.84
Model 2[d]	*Ref*	1.11 (0.83, 1.50)	1.10 (0.77, 1.57)	0.94 (0.61, 1.43)	0.72	0.52
Model 3[e]	*Ref*	1.13 (0.84, 1.52)	1.09 (0.76, 1.55)	0.87 (0.57, 1.32)	0.48	0.27

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; IMT, intima-media thickness.

[a] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to handle model convergence issues. One serving of fruit juices was defined as one medium glass (237 mL).

[b] Computed by assigning the median intake of each category to participants in the corresponding category as a continuous variable.

[c] Adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), BMI (continuous), smoking status (never, past, or current), non-occupational physical activity level (continuous), menopausal status (premenopausal or early perimenopausal), and use of hormone therapy from baseline to the visit of the carotid scan (ever or never). The baseline covariates were used unless specified otherwise.

[d] Model 1 + dietary covariates: The dietary covariates included total energy intake, Alternate Healthy Eating Index, and intake of whole milk, all of which were continuous and the average values of baseline and Visit 5.

[e] Model 2 + physiological risk factors: The physiological risk factors included elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol, all binary and measured at baseline.

Supplemental Table 2.7 Average intake of milk with lower fat content and subclinical carotid atherosclerosis among 931 participants of the Study of Women's Health Across the Nation[a]

Milk with lower fat content, servings/d					*P*	*P*-trend[b]
	None	> 0 to 0.5	> 0.5 to 1	> 1		
n (%)	94 (10.10)	362 (38.88)	198 (21.27)	277 (29.75)		
CCA-IMT, mm						
Model 1[c]	0.949 (0.920, 0.978)	0.951 (0.931, 0.971)	0.939 (0.916, 0.963)	0.938 (0.916, 0.961)	0.61	0.24
Model 2[d]	0.949 (0.920, 0.980)	0.950 (0.930, 0.971)	0.940 (0.917, 0.964)	0.940 (0.917, 0.962)	0.71	0.31
Model 3[e]	0.959 (0.928, 0.990)	0.964 (0.942, 0.986)	0.953 (0.928, 0.978)	0.953 (0.928, 0.977)	0.71	0.33
CCA-AD, mm						
Model 1[c]	7.37 (7.24, 7.51)	7.28 (7.18, 7.37)	7.24 (7.13, 7.35)	7.21 (7.10, 7.31)[f]	0.13	0.047
Model 2[d]	7.37 (7.23, 7.51)	7.27 (7.18, 7.37)	7.24 (7.13, 7.35)	7.22 (7.11, 7.32)[f]	0.23	0.10
Model 3[e]	7.42 (7.27, 7.56)	7.34 (7.23, 7.44)	7.30 (7.18, 7.42)	7.28 (7.16, 7.39)	0.27	0.10
Carotid plaque						
Model 1[c]	*Ref*	1.08 (0.73, 1.61)	0.92 (0.59, 1.44)	1.07 (0.71, 1.61)	0.73	0.92
Model 2[d]	*Ref*	1.10 (0.73, 1.66)	0.93 (0.59, 1.47)	1.04 (0.69, 1.59)	0.72	0.80
Model 3[e]	*Ref*	1.14 (0.76, 1.71)	0.98 (0.62, 1.55)	1.08 (0.71, 1.65)	0.75	0.87

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; IMT, intima-media thickness.

[a] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to handle model convergence issues. One serving of milk with lower fat content was defined as one medium glass (237 mL).

[b] Computed by assigning the median intake of each category to participants in the corresponding category as a continuous variable.

[c] Adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), BMI (continuous), smoking status (never, past, or current), non-occupational physical activity level (continuous), menopausal status (premenopausal or early perimenopausal), and use of hormone therapy from baseline to the visit of the carotid scan (ever or never). The baseline covariates were used unless specified otherwise.

[d] Model 1 + dietary covariates: The dietary covariates included total energy intake, Alternate Healthy Eating Index, and intake of whole milk, all of which were continuous and the average values of baseline and Visit 5.

[e] Model 2 + physiological risk factors: The physiological risk factors included elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol, all binary and measured at baseline.

[f] $P<0.05$ (compared to the reference group).

Chapter 3. Prospective Associations Between Dietary Patterns During the Midlife Derived by Multiple Statistical Methods and Subclinical Carotid Atherosclerosis

3.1 Abstract

Background: Menopause has adverse effects on cardiometabolic profiles that are linked to an increased risk of atherosclerosis and cardiovascular disease in women. A healthy diet during the menopausal transition may counteract the menopause-induced atherosclerotic risk. However, the associations between dietary patterns during the midlife and atherosclerosis later in life among women are unclear.

Methods: A total of 1,246 midlife women from the Study of Women's Health Across the Nation who completed dietary assessments and had a carotid ultrasound scan were used to evaluate the prospective associations of empirically derived dietary patterns with subclinical carotid atherosclerosis. Dietary data were collected at three time points, during 1996-1997, 2001-2003, and 2005-2007. Measures of carotid atherosclerosis and remodeling included common carotid artery intima-media thickness, adventitial diameter, and carotid plaque index collected during 2009-2013. Three statistical methods, including principal component analysis, reduced rank regression, and partial least squares regression, were used to identify dietary patterns. C-reactive protein and plasminogen activator inhibitor 1 were used as the intermediate response variables for reduced rank regression and partial least squares regression.

Results: We identified a Western dietary pattern from each method and a Prudent dietary pattern from the principal component analysis. Adherences to the three Western patterns were all positively associated with intima-media thickness (*P*-trend: 0.013, 0.0058, and < 0.001, for principal component analysis, reduced rank regression, and partial least squares regression, respectively). The Prudent pattern was not significantly associated with intima-media thickness. No associations were found between the identified dietary patterns and adventitial diameter or carotid plaque.

Conclusions: The positive association between the Western diet and intima-media thickness was robust under different dietary pattern derivation methods. The adoption of a diet low in red meat, processed meat, deep fried products, and sugar-sweetened beverages among midlife women may protect against future atherosclerosis.

3.2 Introduction

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in the United States.[1] Although deaths attributable to CVD have been declining, CVD still claims more lives than cancer and chronic lower respiratory disease combined.[1] Over and above the effects of chronological aging, menopause has unfavorable effects on cardiometabolic profiles in women, including increases in visceral fat and total abdominal fat,[4, 5] and elevations in total cholesterol, low-density lipoprotein (LDL) cholesterol, and apolipoprotein B.[6, 104-106] Potentially due to these adverse alterations in cardiometabolic factors, menopause is associated with an accelerated progression of atherosclerosis[11, 26] and an elevated risk of clinical CVD[27] in women.

Diet is a major modifiable risk factor for CVD. The classic "diet-heart" hypothesis states that high dietary intake of cholesterol and saturated fats and low intake of polyunsaturated fats cause an increase in serum cholesterol level, which then leads to the development of atherosclerosis.[58] The traditional approach of nutritional epidemiology focuses on the potential effects of individual foods or nutrients.[57] Dietary pattern analysis is a complementary approach that allows for the examination of the overall diet. Dietary pattern analysis is less subject to the several methodological limitations common in the traditional "single food/nutrient" approach (e.g., small effects of individual foods, complicated interaction among nutrients, and mutual confounding between dietary exposures). Results from dietary pattern analyses can also be more directly translated into dietary guidelines.[57, 58]

The cardiovascular risk induced by menopause may be counteracted by the adoption of a healthy dietary pattern during the menopausal transition.[26, 107] However, the association between diet during the midlife and atherosclerosis later in life among women is inconclusive,[59-67] which is likely partially due to certain methodological issues. Most prior studies used dietary data

collected at a single time point to derive dietary patterns. As an individual's dietary intake may change over time, only one dietary measurement may not accurately capture the long-term dietary habits.[68, 69] Multiple variable reduction techniques are available for dietary pattern identification,[71, 74] which can also potentially lead to inconsistent results. Principal component analysis is the most commonly used method for empirical dietary pattern analysis.[71] Reduced rank regression and partial least squares regression are alternative techniques and allow for the incorporation of *a priori* hypothesis of potential pathophysiological pathways in dietary patterns identification.[71, 74, 75] However, few studies have compared the performances of different variable reduction techniques.[75-77]

The midlife is a crucial period for cardiovascular risk prevention in women, and midlife women may benefit considerably from targeted dietary interventions. Therefore, the potential effects of dietary patterns in this understudied group warrant further investigations. We aimed to: 1) use repeatedly collected dietary data from the Study of Women's Health Across the Nation (SWAN) to evaluate the prospective associations between dietary patterns during the midlife and measures of subclinical carotid atherosclerosis later in life; and 2) compare the performances of three variable reduction techniques in the estimation of diet-disease associations. The three variable reduction techniques included principal component analysis, reduced rank regression, and partial least squares regression.

3.3 Methods

3.3.1 Study Design and Study Population

The SWAN is a multicenter, multiethnic, prospective cohort study initiated in 1996 to study the natural history of menopause.[87] The participants were recruited from seven sites including Boston, Chicago, Southeastern Michigan, Los Angeles, Newark (New Jersey), Pittsburgh, and Oakland (California). Participants were women who self-identified as African American (Pittsburgh, Chicago, Detroit, and Boston), Chinese (Oakland), Japanese (Los Angeles), Hispanic (Newark), or non-Hispanic white (all sites). Baseline eligibility criteria included age 42 to 52 years, having an intact uterus and at least one ovary, not pregnant or lactating, not using oral contraceptives or hormone therapy in the past three months, and having at least one menstrual cycle in the past three months. At baseline, 3,302 women were enrolled. Clinic assessments began in 1996 and participants have been followed up for 15 examinations conducted approximately annually, most recently in 2015-2016. The SWAN protocols were approved by the Institutional Review Board at each site. All participants provided written informed consent at each study visit.

Carotid ultrasound scans were performed at all sites except the Los Angeles site at follow-up Visit 12 (2009-2011) or Visit 13 (2011-2013), with the vast majority of scans conducted at Visit 12. Among the 2,806 women initially enrolled at the six sites, 1,990 (70.9%) participants attended Visit 12, 1,592 (80.0%) of whom had a carotid scan at Visit 12 or Visit 13. An additional 14 women did not attend Visit 12, but attended and received the carotid scan at Visit 13. Thus, a total of 1,606 women had a carotid scan. For this analysis, we excluded women who had incomplete data on the three specific measures of carotid atherosclerosis (n = 54); who

did not have high-quality dietary data at any visit (defined as not reporting too few [< 4/day] or too many [> 16/day] solid foods, not skipping more than 10 food items on the questionnaire, and not reporting total energy intake that was too low [< 2,092 kJ/day or 500 kcal/day] or too high [>20,920 kJ/day or 5,000 kcal/day]) (n = 17); who self-reported having heart disease (n = 51) or stroke (n = 9) at baseline or developed heart disease (n = 38) or stroke (n = 35) during the follow-up before their carotid scans; and who had missing data for the major covariates including education level, financial strain, self-rated overall health, smoking status, non-occupational physical activity level, menopausal status, abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced high-density lipoprotein (HDL) cholesterol (n = 156). After the aforementioned exclusions, the final analytical sample for principal component analysis consisted of 1,246 women. Additionally, 231 women had missing data on the intermediate response variables (C-reactive protein and thrombotic marker plasminogen activator inhibitor 1), so the final analytical sample for reduced rank regression and partial least squares regression included 1,015 women (**Figure 3.1**). Compared to the excluded participants, the women retained in the analysis were *less* likely to be African American, to report difficulty financial strain, to have depressive symptoms, and to have abdominal obesity, elevated blood pressure, elevated fasting glucose, and reduced HDL cholesterol. The retained participants were *more* likely to have a college degree and to self-report having excellent or very good overall health.

3.3.2 Assessment of Exposures

Dietary intake was measured at baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007). We used a modified 1995 version of the Block food frequency questionnaire, which has previously been validated against dietary records[88] and 24-hour recalls.[89] The questionnaire included 103 food items. Trained personnel asked the participants how often they consumed each food item during the past year, as well as the usual portion size for each item. Up to nine predefined frequencies (ranging from never to ≥5 times/day) and three predefined portion sizes (ranging from small to large) were available for each item. Total energy intake and nutrient intakes were calculated by multiplying the reported frequency, the reported portion size, and the corresponding nutrient content using the DIETSYS software.[108] We aggregated the 103 foods into 46 prespecified groups based on nutrient profile or culinary use (**Supplemental Table 3.1**). We calculated the intake (in grams) for each food group and adjusted it for total energy intake using the residual method.[109] We then computed the energy-adjusted food group values averaged across up to three available dietary measurements (baseline, Visit 5, and Visit 9) to capture the long-term intakes; 63.6%, 25.4%, and 10.9% of the participants had three, two, and one available dietary measurements, respectively.

3.3.3 Assessment of Outcomes

Centrally trained and certified sonographers obtained carotid ultrasound images at Visit 12 or Visit 13 using a Terason t3000 Ultrasound System (Teratech Corp, Burlington, Massachusetts) with a variable frequency linear array transducer.[90] Two digitized images were obtained for each of the left and right distal common carotid artery (CCA). From each of these four images, near and far wall intima-media thickness (IMT) measures of the CCA were

obtained by electronically tracing the lumen-intima interface and the media-adventitia interface across a 1-cm segment proximal to the carotid bulb. The mean of the maximal readings of all four images was used in the analyses. Adventitial diameter (AD) was measured as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interface on the far wall at end-diastole across the same CCA segments used for IMT measurement. The mean of the average readings was used in the analyses. The presence and extent of plaque in each of five segments of the left and right carotid artery (distal and proximal CCA, carotid bulb, and proximal internal and external carotid arteries) was evaluated. The degree of the plaque for each segment was graded between 0 (no observable plaque) and 3 (plaque obstructing ≥ 50% of the luminal diameter). The grades from all segments were summed to create the plaque index.[91] All carotid scan images were read centrally at the SWAN Ultrasound Reading Center at the University of Pittsburgh. The outcomes of this study included the intima-media thickness of the common carotid artery (CCA-IMT), the adventitial diameter of the common carotid artery (CCA-AD), and the carotid plaque index. We treated CCA-IMT and CCA-AD as continuous variables and carotid plaque index as a binary variable (≥ 2 versus < 2). CCA-IMT and CCA-AD had fairly normal distributions, and no transformations were performed.

3.3.4 Assessment of Covariates

Self-reported baseline covariates included age, race/ethnicity, education level (≤ high school, some college, or college degree/post-college), financial strain (dichotomized by the difficulty paying for basic necessities), self-rated overall health (excellent/very good, good, or fair/poor), depressive symptoms (dichotomized by the Center for Epidemiologic Studies

Depression Scale: ≥16 or <16),[92] smoking status (never, past, or current), non-occupational physical activity (assessed on five-point Likert-scale questions with total scores ranging from 3 to 15),[93] and menopausal status based on self-reported menstrual bleeding patterns (dichotomized as premenopausal or early perimenopausal). Self-reported use of hormone therapy from baseline through the visit of the carotid scan was dichotomized as ever use or never use, with ever use defined as the use of hormone therapy at any visit from baseline to the visit of the carotid scan. Waist circumference was measured with a measuring tape placed horizontally at the level of the natural waist or the narrowest part of the torso from the anterior aspect. Blood pressure was calculated as the average of two seated measurements using a standard mercury sphygmomanometer. Blood samples were taken to measure fasting glucose and blood lipids. Cardiometabolic conditions were defined using established harmonized guidelines.[96] Specifically, abdominal obesity was defined as waist circumference ≥ 80 cm for Chinese women and ≥ 88 cm for others. Elevated blood pressure was defined as systolic blood pressure ≥ 130 mm Hg, or diastolic blood pressure ≥ 85 mm Hg, or antihypertensive drug treatment. Elevated fasting glucose was defined as fasting glucose ≥ 100 mg/dL or drug treatment of elevated glucose. Elevated triglycerides was defined as fasting serum triglycerides ≥ 150 mg/dL. Reduced HDL cholesterol was defined as fasting serum HDL cholesterol < 50 mg/dL.

3.3.5 Statistical Analysis

We used three complementary statistical techniques, including principal component analysis (PCA), reduced rank regression (RRR), and partial least squares regression (PLS), to identify dietary patterns. PCA combines correlated food items into weighted linear combinations

(i.e., dietary patterns) that account for maximum variation of the original food variables.[71] RRR aims to identify food combinations that explain the maximum variation in a set of prespecified intermediate response variables, and PLS aims to derive food combinations that explain variation in both the intermediate response variables and the food variables.[74] The intermediate response variables are the potential mediators for the diet-disease association based on *a priori* hypotheses. Because of their abilities to incorporate the intermediate response variables, RRR and PLS can be used to explore potential biological mechanisms for the diet-disease associations.

We used the 46 energy-adjusted food groups (averaged across up to three visits) as the inputs of PCA, RRR, and PLS. Inflammatory marker C-reactive protein (CRP) and thrombotic marker plasminogen activator inhibitor 1 (PAI-1) were used as the intermediate response variables for RRR and PLS. We chose CRP and PAI-1 because the effect of diet on subclinical atherosclerosis may be partly mediated by pro-inflammatory and pro-thrombotic pathways.[60, 61] We used the values at Visit 6 (2002-2004) for CRP and PAI-1 and natural log-transformed both variables to improve normality. The number of dietary patterns initially derived by PCA and PLS is restricted by the number of food variables used (i.e., 46 in this study), whereas the number of patterns derived by RRR is constrained by the number of intermediate response variables used (i.e., two in this study). The number of patterns to retain from PCA for further analysis was based on the scree plot,[110] the eigenvalue > 1 criterion, and the overall interpretability of the principal components.[111] For RRR and PLS, the first patterns were retained for further analysis as they explained more variation in the intermediate response variables than the succeeding patterns. We calculated the factor scores for each retained pattern where a higher score represented a higher adherence to the corresponding pattern. We computed the correlation

coefficients between the intermediate response variables and the factor scores, and between the intermediate response variables and the food groups.

We estimated the associations of quartiles of dietary pattern factor scores with CCA-IMT/CCA-AD using linear models and with high carotid plaque index (≥ 2) using log-binomial models (using the first quartile as the reference group). We used modified Poisson models with robust variance estimation to achieve model convergence.[97] Least squares means and 95% confidence intervals (CIs) of CCA-IMT/CCA-AD (unit: mm) were estimated from the linear models. Risk ratios and 95% CIs of high carotid plaque index were estimated from the modified Poisson models. The selection of confounders was based on prior knowledge of CVD risk factors. We adjusted for age, race/ethnicity, education level, financial strain, self-rated overall health, depressive symptoms, smoking status, non-occupational physical activity level, total energy intake, menopausal status, use of hormone therapy, abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, reduced HDL cholesterol, and the number of missing visits for dietary measurements. All covariates were the baseline values except age (at the carotid scan), total energy intake (average across available visits of baseline, Visit 5, and Visit 9), hormone therapy use (ever use from baseline to the visit of the scan), and the number of missing visits for dietary measurements (0, 1, or 2 based on data availability across baseline, Visit 5, and Visit 9). To test for linear trends, we assigned the median factor score of each quartile to participants in the corresponding quartile as a continuous variable in the models.

We conducted four sensitivity analyses to examine the robustness of the results. First, to assess potential selection bias due to missing data, we used inverse probability weighting to incorporate a non-response weight for each participant based on her baseline predictors of

attrition including race, education level, financial strain, self-rated overall health, depressive symptoms, smoking status, physical activity level, menopausal status, abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol. Second, we used the average food groups of baseline and Visit 5 to derive dietary patterns (i.e., ignoring dietary data at Visit 9). The motivation of this sensitivity analysis was to assess whether the inclusion of the Visit-9 dietary data appreciably influenced the estimates as the intermediate response variables were the Visit-6 values due to data availability (PAI-1 data were not available after Visit 9). Third, we derived dietary patterns *separately* for baseline and Visit 5 using the food groups at each visit and then used the average factor scores (in quartiles) in the analyses. Fourth, to examine potential time-varying confounding by variables that are confounders for future exposures but mediators for past exposures, we used the inverse-probability-of-treatment weighting and marginal structural models[112] to additionally adjust for cardiometabolic outcomes measured at Visit 3 (1999-2001), including abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced HDL cholesterol. All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC) at a two-sided alpha level of 0.05.

3.4 Results

The general characteristics of the analytical sample are presented in **Table 3.1**. The mean age of the participants at baseline was 46.3 years with a standard deviation (SD) of 2.6 years. Approximately half of the participants (52.8%) were non-Hispanic white, 28.6% were African American, 12.7% were Chinese, and 5.9% were Hispanic. At baseline, 62.2% of the participants were never smokers, 25.7% were past smokers, and 12.1% were current smokers. The mean CCA-IMT and CCA-AD at the visit of the carotid scan were 0.92 mm (SD: 0.14) and 7.19 mm (SD: 0.65), respectively, and 25.3% of the participants had high carotid plaque index.

Two dietary patterns were retained from PCA, which jointly explained 17.20% of the variation in food intake but only 2.35% of the variation in the intermediate response variables. The first pattern was named the PCA-Western pattern and the second pattern was named the PCA-Prudent pattern. The PCA-Western pattern explained 10.71%, 1.43%, and 0.00% of the variation in food intake, CRP, and PAI-1, respectively. The PCA-Prudent pattern explained 6.49%, 2.07%, and 1.21% of the variation in food intake, CRP, and PAI-1, respectively (**Table 3.2**). The PCA-Western pattern was characterized by high intakes of dairy products, pizza, unprocessed red meat, processed red meat, and salad dressings. The PCA-Prudent pattern included high intakes of vegetables, legumes, and fruits. The PCA-Western pattern was weakly correlated with a higher level of CRP (Spearman correlation coefficient: 0.10). The PCA-Prudent pattern was weakly correlated with lower levels of CRP (Spearman correlation coefficient: -0.18) and PAI-1 (Spearman correlation coefficient: -0.09) (**Table 3.3**).

Only the first patterns from RRR and PLS were retained for further analyses as they explained more variation in the intermediate response variables. These two patterns were consequently named the RRR-Western pattern and the PLS-Western pattern, respectively. The

RRR-Western pattern explained 3.65%, 12.45%, and 4.20% of the variation in food intake, CRP, and PAI-1, respectively. The PLS-Western pattern explained 8.06%, 8.41%, and 1.89% of the variation in food intake, CRP, and PAI-1, respectively (**Table 3.2**). The RRR-Western pattern was characterized by high intakes of margarine, fried fish, artificially sweetened beverages, processed red meat, and sugar-sweetened beverages, and low intakes of soup, fiber cereals, fruits, cruciferous vegetables, and wine. The PLS-Western pattern included high intakes of French fries, processed red meat, fried fish, margarine, and sugar-sweetened beverages, and low intakes of fruits, legumes, dark-yellow vegetables, cruciferous vegetables, and soup. Both the RRR-Western pattern and the PLS-Western pattern were positively correlated with CRP (Spearman correlation coefficient: 0.39 and 0.32, respectively) and PAI-1 (Spearman correlation coefficient: 0.22 and 0.15, respectively) (**Table 3.3**). Descriptive statistics of the covariates by quartiles of all four dietary pattern factor scores are provided in **Supplemental Table 3.2**. The retained patterns are graphically presented in **Figure 3.2**, which visually shows that the PCA-Prudent pattern is distinct from the three Western patterns while the Western patterns are somewhat similar to one another.

Adherences to PCA-Western, RRR-Western, and PLS-Western patterns were all positively associated with CCA-IMT after adjusting for covariates (*P*-trend: 0.013, 0.0058, and < 0.001, respectively) (**Table 3.4**). Women in the fourth quartile of the PCA-Western, the RRR-Western, and the PLS-Western patterns had 0.042 mm (95% CI: 0.011, 0.073), 0.033 mm (95% CI: 0.009, 0.057), and 0.049 mm (95% CI: 0.025, 0.074), respectively, larger CCA-IMT than women in the first quartile; these differences correspond to 30%, 24%, and 35% of the sample SD of CCA-IMT, respectively. Adherence to the PCA-Prudent pattern was not significantly

associated with CCA-IMT (*P*-trend = 0.38). We found no associations between any identified dietary patterns and CCA-AD or carotid plaque.

The results did not change appreciably after accounting for missing data using non-response weights, using the average food groups of baseline and Visit 5 to derive dietary patterns, deriving dietary patterns separately for baseline and Visit 5, or adjusting for time-varying confounders using marginal structural models. Results from the sensitivity analyses are shown in **Supplemental Tables 3.3-3.5**.

3.5 Discussion

This study evaluated the prospective associations between empirically derived dietary patterns during the midlife and subclinical carotid atherosclerosis later in life among women. After extensively adjusting for covariates, the Western dietary patterns derived by PCA, RRR, and PLS were all positively associated with CCA-IMT. The Prudent pattern was not significantly associated with CCA-IMT, and no significant associations were found between the identified dietary patterns and CCA-AD or carotid plaque.

Menopause has unfavorable effects on cardiometabolic profiles which are linked to an elevated risk of atherosclerosis and CVD in women.[4-6, 104-106] Adherence to a healthy dietary pattern during the menopausal transition may somewhat offset the menopause-induced cardiovascular risk.[26, 107] The literature on dietary patterns and subclinical atherosclerosis is inconclusive with some studies suggesting the presence of an association[59-63] and others finding no such evidence.[64-67] Moreover, few studies have specifically examined the associations between dietary patterns during the midlife and atherosclerosis later in life among women. SWAN followed women from premenopause into postmenopause with dietary data repeatedly collected throughout the transition, which provides a valuable opportunity to characterize this important yet understudied association.

In the Framingham Study, women (mean age approximately 47 years at baseline) with a cluster-analysis-derived dietary pattern high in fat and sugar and low in fruit and vegetables had significantly higher odds of carotid artery stenosis after 12 years compared to women with a "Heart-Healthy" diet at baseline.[59] In a cross-sectional study in Spain using cluster analysis (participants aged 40 to 54 years; 37% women), compared to participants following the Mediterranean diet, those with a "social-business" diet high in red meat, pre-made foods, snacks,

and sugar-sweetened beverages had significantly higher odds of coronary artery calcification.[63] As a counterexample, in a Finnish cohort of participants aged 24 to 39 years at baseline (54% women), a PCA-derived pattern with high intakes of rye, potatoes, butter, sausages, milk, and coffee was *not* associated with CCA-IMT in women 21 years later.[65] In a French cohort of women aged 35 to 60 years at baseline, *none* of the four PCA-derived patterns, including a Western pattern high in processed meat and a Prudent pattern high in fruits, vegetable, and fish, were significantly associated with CCA-IMT or carotid plaque measured 7.5 years later.[66] All but one[65] of the studies mentioned above used a single dietary assessment to measure diet thus were unlikely to have accurately captured the long-term diet. Furthermore, all four studies included almost exclusively white participants, so the generalizability of the results to other racial and ethnic groups may be limited.

The use of different dietary pattern derivation techniques and intermediate response variables may partially explain the inconsistency in the literature. PCA uses the correlations among food variables, and the derived patterns are heavily influenced by cultural backgrounds. RRR and PLS allow for the incorporation of intermediate variables thus may assess potential etiological pathways and inform prevention strategies targeted toward specific pathways. RRR has gained popularity in nutritional epidemiology recently, but few studies have compared the performances of RRR (and PLS) to the more traditionally used PCA.[75-77]

Two prior studies have used RRR (but not PLS) to evaluate the association between dietary patterns and subclinical atherosclerosis. In a cross-sectional analysis of the Multi-Ethnic Study of Atherosclerosis, an RRR-derived pattern high in total and saturated fat and low in fiber and micronutrients was associated with higher CCA-IMT and coronary artery calcification.[60] Similarly, in the Insulin Resistance Atherosclerosis Study, a baseline RRR-derived pattern with

high intakes of refined grains, red meat, cheese, and sugar-sweetened beverages was positively associated with CCA-IMT after five years.[61]

The association we observed between the RRR-derived pattern and CCA-IMT was consistent with prior studies, although we used somewhat different intermediate response variables (CRP and PAI-1) than those used in the Multi-Ethnic Study of Atherosclerosis (CRP, interleukin-6, homocysteine, and fibrinogen) and the Insulin Resistance Atherosclerosis Study (PAI-1 and fibrinogen). CRP as an inflammatory marker and PAI-1 as a thrombotic marker are both potential markers for CVD risk.[113, 114] Further, the effect of diet on subclinical atherosclerosis may be partly mediated by pro-inflammatory and pro-thrombotic processes.[60, 61] Contrary to the results from the Multi-Ethnic Study of Atherosclerosis, which reported that a PCA-derived Western pattern was not associated with CCA-IMT,[60] the PCA-Western pattern in the present study was associated with CCA-IMT with a magnitude comparable to that for the RRR-Western pattern despite the fact that the PCA-Western pattern explained little variation in the intermediate response variables. Therefore, it is likely that the PCA-Western pattern may promote atherosclerosis independent of the inflammation or hemostasis processes, or through alternative inflammatory and hemostatic mechanisms that are not well accounted for by CRP or PAI-1. Other potential inflammatory and hemostatic intermediate response variables we considered included interleukin 6, homocysteine, and fibrinogen, which were unavailable in SWAN. We also used traditional cardiometabolic risk factors (waist-to-hip ratio, systolic blood pressure, triglycerides, HDL cholesterol, and fasting glucose) as the intermediate response variables, and found that the resultant dietary patterns were not significantly associated with subclinical carotid atherosclerosis (results not shown). This null finding is consistent with Liese et al.,[61] who also initially used traditional cardiovascular risk factors as the intermediate response

variables of RRR and found that only the selection of PAI-1 and fibrinogen yielded dietary patterns that were associated with CCA-IMT. Therefore, our study provides evidence that the effect of diet on subclinical carotid atherosclerosis may be mediated by inflammatory and hemostatic mechanisms rather than through traditional cardiometabolic risk factors.

Among the three Western patterns in this study, the PLS-Western pattern has the strongest association with CCA-IMT. PLS is a compromise between PCA and RRR, with the capability of identifying food combinations that are not only commonly consumed in the population but also associated with intermediate risk factors.[74] PLS offers more flexibility over RRR, especially in exploratory analyses in the presence of uncertainty about the mechanistic pathways.[75] PLS has been underutilized in the nutritional epidemiologic literature. Future studies can take advantage of this relatively novel technique to uncover the interrelationships among dietary exposures, intermediate risk factors, and disease outcomes.

In the present study, compared to being in the lowest quartile, being in the highest quartile of the Western patterns was associated with differences in CCA-IMT equivalent to 24% to 35% of the sample SD. Previous studies report that every one-SD increment in CCA-IMT is associated with a 26% higher risk of myocardial infarction and a 32% higher risk of stroke.[15] While the effect estimates from the present study may seem small in magnitude, they are of public health relevance due to the high prevalence of Western diet in the United States. Also, the estimates from the present study may be conservative as the women retained in the analysis were generally healthier compared to the baseline sample of women who were initially enrolled in SWAN. While it was not possible to pinpoint the nutrients that were most responsible for the observed associations, all three Western patterns were characterized by high intakes of foods rich in saturated fat,[115] *trans* fat,[115] or cholesterol,[116] which have all been associated with larger CCA-

IMT. Interestingly, the Prudent dietary pattern *per se*, with relatively high loadings on fruits, vegetables, and legumes, is not associated with subclinical atherosclerosis in this study. This lack of association may be explained by the overall low intakes of healthy foods and the relatively narrower distribution of the Prudent pattern factor scores compared to the three Western patterns.

The primary strengths of this study include the focus on multiethnic midlife women, the comparison of multiple data reduction methods, the comprehensive adjustment of covariates, and the use of multiple prospective measures of dietary intake to reduce random within-person variation in diet and to more accurately capture the long-term diet.[58] This study also has some limitations. First and foremost, as carotid atherosclerosis was measured only once, we were unable to evaluate the change of subclinical atherosclerosis over time. Second, the self-reported dietary intake inevitably had a certain degree of measurement error, which was minimized by the use of up to three repeated measures from validated food frequency questionnaires and by the restriction to women with high-quality dietary data. Also, the validity of a dietary pattern analysis does not depend on the accurate quantification of the absolute intake, but the relative ranking of the individuals; the food frequency questionnaire is relatively robust in ranking the levels of dietary intake.[58] Third, CRP and PAI-1 may not represent the inflammatory and hemostatic processes that are most pertinent to the development of atherosclerosis, in which case the patterns derived by RRR and PLS may underestimate the true associations. Fourth, this study was subject to multiple testing due to the inclusion of multiple dietary patterns and outcomes. After controlling for the false discovery rate of the 12 exposure-outcome combinations,[103] the associations between the three Western patterns and CCA-IMT remained significant or marginally significant (adjusted *P*-trend: 0.052, 0.035, and 0.0012 for PCA-Western, RRR-Western, and PLS-Western patterns, respectively). The association between the PLS-Western

pattern and CCA-IMT remained significant also under the Bonferroni correction (adjusted *P*-trend = 0.0012). Furthermore, the results from all analyses were reported, and the key findings did not change considerably under various sensitivity analyses.

In conclusion, this prospective study identifies three Western dietary patterns that are associated with more subclinical carotid atherosclerosis in midlife women. The menopausal transition in women represents a vulnerable window of increased cardiovascular risk and a crucial period for CVD prevention. The results from this study indicate that the positive association between the Western diet and intima-media thickness of the common carotid artery is robust under various statistical methods of dietary pattern identification. The adoption of a diet low in red meat, processed meat, deep fried products, and sugar-sweetened beverages among midlife women may protect against future atherosclerosis.

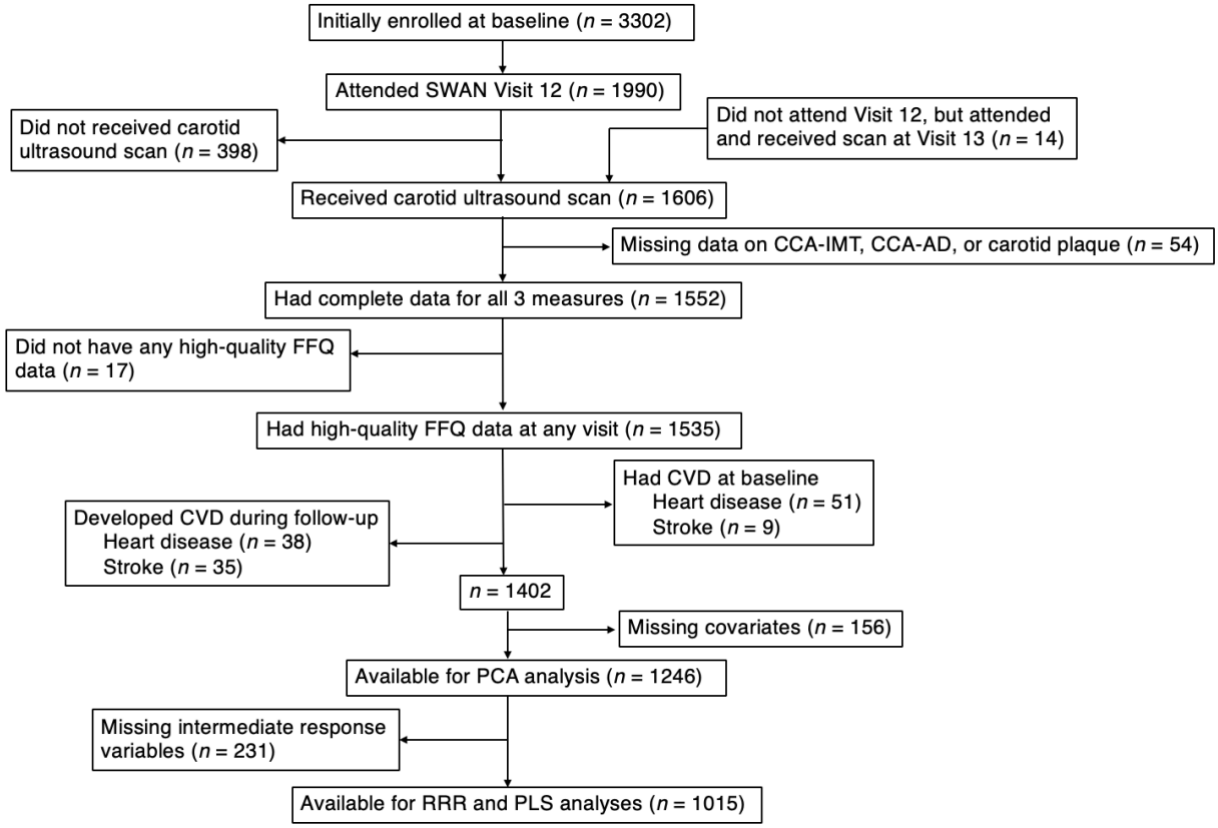

Figure 3.1 Exclusion flow of participants for the association between dietary patterns and subclinical carotid atherosclerosis in the Study of Women's Health Across the Nation. High-quality food frequency questionnaire data was defined as not reporting too few (< 4/day) or too many (> 16/day) solid foods, not skipping more than 10 food items on the questionnaire, and not reporting total energy intake that was too low (< 2,092 kJ/day or 500 kcal/day) or too high (>20,920 kJ/day or 5,000 kcal/day). Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CVD; cardiovascular disease; FFQ, food frequency questionnaire; IMT, intima-media thickness.

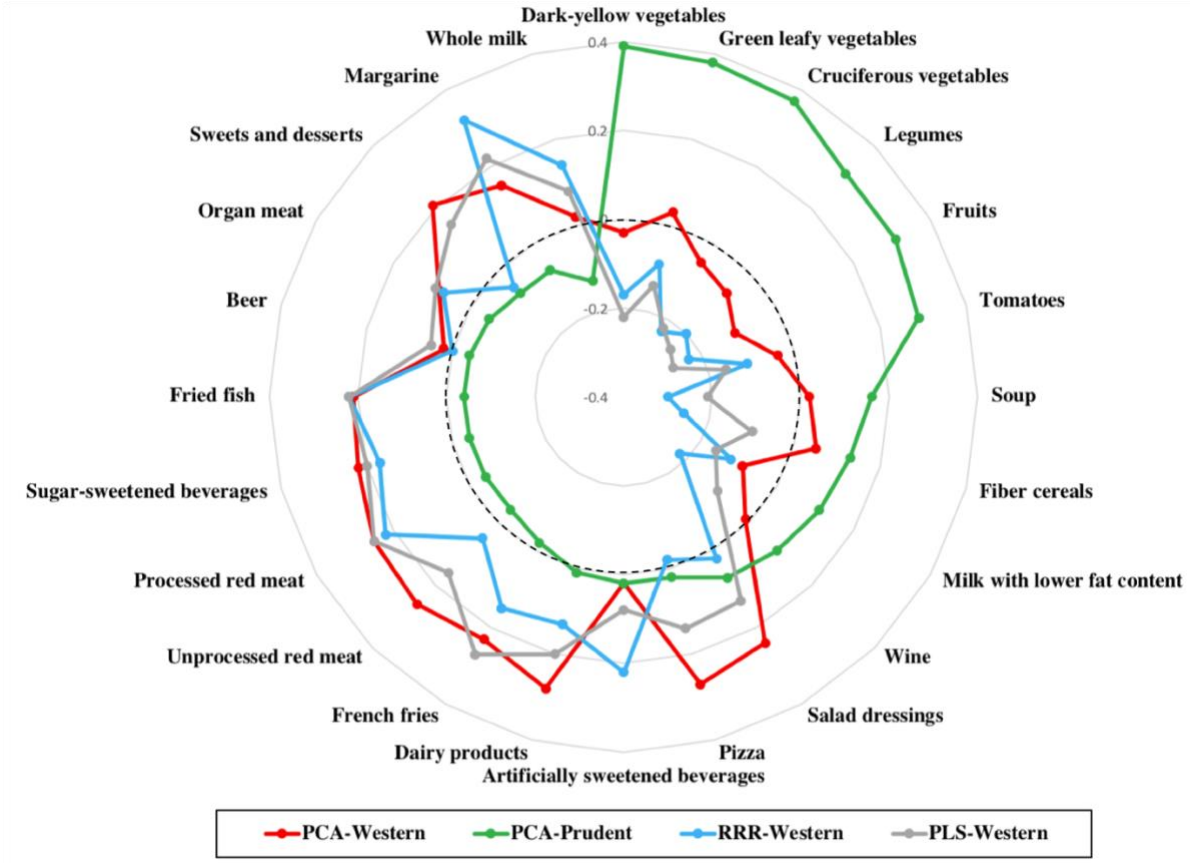

Figure 3.2 Dietary patterns derived using three statistical methods among participants in the Study of Women's Health Across the Nation (n = 1,246 for principal component analysis, and n = 1,015 for reduced rank regression and partial least squares regression). The dietary patterns were derived using the energy-adjusted food groups averaged across available visits of baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007). The intermediate response variables for reduced rank regression and partial least squares regression were the log-transformed C-reactive protein and plasminogen activator inhibitor 1 at Visit 6 (2002-2004). Each arm of the shape represents the factor loading of a food group on a dietary pattern. The outer edge of the constellation represents the most positive factor loading (i.e., a strong positive correlation), and the midpoint of the constellation represents the most negative factor loading (i.e., a strong inverse correlation). A factor loading of zero is indicated as the black dashed circle. Abbreviations: PCA, principal component analysis; PLS, partial least squares regression; RRR, reduced rank regression.

Table 3.1 General characteristics of the 1,246 participants included for the association between dietary patterns and subclinical carotid atherosclerosis in the Study of Women's Health Across the Nation[a]

General characteristics	*n* = 1246
Age at baseline, year, mean (SD)	46.3 (2.6)
Age at the carotid scan, year, mean (SD)	60.2 (2.7)
Race and ethnicity	
African American, %	28.6
Hispanic, %	5.9
Chinese, %	12.7
Non-Hispanic white, %	52.8
Education level	
High school or less, %	21.0
Some college, %	30.3
College degree/post-college, %	48.7
Somewhat/very hard to pay for basics, %	32.3
Self-rated overall health	
Excellent/very good, %	63.5
Good, %	27.4
Fair/poor, %	9.2
Depressive symptoms[b], %	22.6
Total energy intake, kcal/day[c], mean (SD)	1751.1 (560.5)
Number of missing dietary measurements	
0, %	63.6
1, %	25.4
2, %	10.9
Smoking status	
Never, %	62.2
Past, %	25.7
Current, %	12.1
Non-occupational physical activity[d], mean (SD)	7.7 (1.8)
Menopausal status	
Early perimenopausal, %	44.4
Premenopausal, %	55.6
Hormone therapy use[e], %	43.2
Abdominal obesity, %	39.0
Elevated blood pressure, %	26.5
Elevated fasting glucose, %	21.4
Elevated triglycerides, %	17.4
Reduced HDL cholesterol, %	33.3
CCA-IMT, mm[f], mean (SD)	0.9 (0.1)
CCA-AD, mm[f], mean (SD)	7.2 (0.7)
High carotid plaque index[f, g], %	25.3

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; HDL, high-density lipoprotein; IMT, intima-media thickness; SD, standard deviation.

[a] Values are means (standard deviations) for continuous variables and percentages for categorical variables. Values of polytomous variables may not sum to 100% due to rounding. The variables are the baseline (1996-1997) measures unless specified otherwise.

[b] Defined as the Center for Epidemiologic Studies Depression Scale ≥ 16.

[c] Average value across available visits of baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007).

[d] Assessed on five-point Likert and ordinal quantitative scales with total scores ranging from 3 to 15, with higher values indicating more frequent engagement in non-occupational physical activity.

[e] Defined as reported use of hormone therapy at any time from baseline to the visit of the carotid scan.

[f] Measured at Visit 12 (2009-2011) or Visit 13 (2011-2013).

[g] Defined as carotid plaque index ≥ 2.

Table 3.2 Percentage of variation in the intermediate response variables and food variables explained by the dietary patterns derived by multiple statistical methods among 1,246 participants of the Study of Women's Health Across the Nation[a]

	Explained variation in intermediate response variables[b], %			Explained variation in food groups, %
	CRP	PAI-1	Both CRP and PAI-1	
PCA				
PCA-Western	1.43	0.00	0.72	10.71
PCA-Prudent	2.07	1.21	1.63	6.49
Both PCA patterns	3.50	1.21	2.35	17.20
RRR-Western	12.45	4.20	8.32	3.65
PLS-Western	8.41	1.89	5.15	8.06

Abbreviations: CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor 1; PCA, principal component analysis; PLS, partial least squares regression; RRR, reduced rank regression.

[a] The dietary patterns were derived using the energy-adjusted food groups averaged across available visits of baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007). The sample size for reduced rank regression and partial least squares regression was 1,015 due to missing data on the intermediate response variables.

[b] The intermediate response variables were the log-transformed values at Visit 6 (2002-2004).

Table 3.3 Correlations among empirically derived dietary patterns, food groups, and intermediate response variables in 1,246 participants of the Study of Women's Health Across the Nation[a]

		Spearman correlation coefficients with intermediate response variables[b]	
	Factor loading	CRP	PAI-1
PCA-Western		0.10	
High			
Dairy products	0.28	0.09	
Pizza	0.27		
Unprocessed red meat	0.26	0.13	
Processed red meat	0.25	0.25	0.08
Salad dressings	0.24		
Low			
Fruits	-0.11	-0.12	
Milk with lower fat content	-0.09	-0.10	
Legumes	-0.07	-0.13	
Cruciferous vegetables	-0.05	-0.12	-0.09
Tomatoes	-0.04	-0.09	
PCA-Prudent		-0.18	-0.09
High			
Dark-yellow vegetables	0.39	-0.07	-0.07
Green leafy vegetables	0.38		
Cruciferous vegetables	0.37	-0.12	-0.09
Legumes	0.31	-0.13	
Fruits	0.31	-0.12	
Low			
Whole milk	-0.13		
Margarine	-0.07	0.21	0.11
Sweets and desserts	-0.07		
Organ meat	-0.05	0.06	
Beer	-0.04	-0.07	
RRR-Western		0.39	0.22
High			
Margarine	0.32	0.20	0.10
Fried fish	0.22	0.15	
Artificially sweetened beverages	0.22	0.19	
Processed red meat	0.22	0.25	0.08
Sugar-sweetened beverages	0.17	0.17	0.09
Low			
Soup	-0.30	-0.14	
Fiber cereals	-0.26	-0.06	-0.13
Fruits	-0.23	-0.13	
Cruciferous vegetables	-0.23	-0.12	-0.09
Wine	-0.22	-0.12	-0.11
PLS-Western		0.32	0.15
High			
French fries	0.27	0.13	
Processed red meat	0.25	0.25	0.08
Fried fish	0.22	0.15	
Margarine	0.22	0.20	0.10
Sugar-sweetened beverages	0.20	0.17	0.09
Low			
Fruits	-0.27	-0.13	
Legumes	-0.25	-0.12	
Dark-yellow vegetables	-0.22	-0.07	-0.07
Cruciferous vegetables	-0.22	-0.12	-0.09
Soup	-0.21	-0.14	

Abbreviations: CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor 1; PCA, principal component analysis; PLS, partial least squares regression; RRR, reduced rank regression.

[a] The food groups were the energy-adjusted values averaged across available visits of baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007). For simplicity, only the five positively associated and the five inversely associated food groups with the largest factor loadings (absolute value) are shown. The sample size for reduced rank regression and partial least squares regression was 1,015 due to missing data on the intermediate response variables.
[b] The intermediate response variables were the log-transformed values at Visit 6 (2002-2004). For simplicity, only statistically significant ($P < 0.05$) correlation coefficients are shown.

Table 3.4 Quartiles of dietary pattern factor scores from multiple statistical methods and subclinical carotid atherosclerosis among 1,246 participants of the Study of Women's Health Across the Nation[a, b]

	Quartile 1		Quartile 2		Quartile 3		Quartile 4		*P*-trend[c]
	LS-mean or RR	95% CI	LS-mean or RR	95% CI	LS-mean or RR	95% CI	LS-mean or RR	95% CI	
CCA-IMT, mm									
PCA-Western	0.923	0.900, 0.946	0.937	0.918, 0.956	0.957[d]	0.939, 0.976	0.964[d]	0.944, 0.985	0.013
PCA-Prudent	0.951	0.933, 0.969	0.955	0.937, 0.974	0.933	0.914, 0.952	0.945	0.926, 0.964	0.38
RRR-Western	0.934	0.909, 0.958	0.937	0.913, 0.961	0.950	0.927, 0.973	0.966[d]	0.945, 0.988	0.0058
PLS-Western	0.924	0.899, 0.948	0.942	0.918, 0.966	0.943	0.919, 0.966	0.973[e]	0.951, 0.994	< 0.001
CCA-AD, mm									
PCA-Western	7.30	7.19, 7.41	7.34	7.25, 7.43	7.39	7.31, 7.48	7.36	7.26, 7.46	0.65
PCA-Prudent	7.35	7.26, 7.43	7.39	7.30, 7.48	7.34	7.24, 7.43	7.33	7.24, 7.42	0.51
RRR-Western	7.33	7.22, 7.45	7.39	7.27, 7.50	7.36	7.25, 7.47	7.37	7.27, 7.48	0.56
PLS-Western	7.31	7.19, 7.42	7.34	7.22, 7.46	7.33	7.22, 7.44	7.42	7.32, 7.52	0.071
Carotid plaque									
PCA-Western	1.00	*Ref*	0.87	0.65, 1.15	0.85	0.61, 1.17	0.79	0.53, 1.17	0.35
PCA-Prudent	1.00	*Ref*	1.14	0.88, 1.47	1.02	0.77, 1.35	1.09	0.82, 1.50	0.74
RRR-Western	1.00	*Ref*	0.77	0.56, 1.05	0.86	0.63, 1.18	1.01	0.75, 1.35	0.79
PLS-Western	1.00	*Ref*	1.17	0.86, 1.58	1.06	0.78, 1.46	1.10	0.80, 1.51	0.66

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CI, confidence interval; IMT, intima-media thickness; LS-mean, least squares mean; PCA, principal component analysis; PLS, partial least squares regression; RR, risk ratio; RRR, reduced rank regression.

[a] The dietary patterns were derived using the energy-adjusted food groups averaged across available visits of baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007). The outcomes were measured at Visit 12 (2009-2011) or Visit 13 (2011-2013). The sample size for reduced rank regression and partial least squares regression was 1,015 due to missing data on the intermediate response variables.

[b] Values for CCA-IMT/CCA-AD are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). Values for carotid plaque are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to achieve model convergence. All models were adjusted for age at the carotid scan (continuous), race/ethnicity (non-Hispanic white or not), education level ($\leq$ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale ($\geq$16 or <16), smoking status (ever or never), non-occupational physical activity level (continuous), total energy intake (continuous; average across available visits of baseline, Visit 5, and Visit 9), menopausal status (premenopausal or early perimenopausal), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, reduced high-density lipoprotein cholesterol, and the number of missing visits for dietary measurements (0, 1, or 2). The baseline covariates were used except age, total energy intake, hormone therapy use, and the number of missing visits for dietary measurements.

[c] Computed by assigning the median factor score of each quartile to participants in the corresponding quartile as a continuous variable.

[d] $P < 0.01$ (compared to Quartile 1).

[e] $P < 0.001$ (compared to Quartile 1).

Supplemental Table 3.1 Food groupings based on nutrient profile and culinary use in the Study of Women's Health Across the Nation

No.	Food group	Food items
1	Processed red meat	Hot dogs Ham, bologna, other lunch meats, regular or made with turkey Sausage or bacon
2	Unprocessed red meat	Hamburgers, cheeseburgers, beef burritos or tacos, at home or in a restaurant Beef, including roasts, steaks, or in stir-fry or sandwiches Pork, including chops, roasts, or in stir-fry
3	Organ meat	Liver, including chicken livers
4	Fried fish	Fried fish or fish sandwich, at home or in a restaurant
5	Fish (not fried)	Tuna, tuna salad, tuna casserole Other fish, broiled or baked
6	Shellfish	Shellfish such as shrimp, crab, and oysters
7	Poultry	Fried chicken, at home or in a restaurant Chicken or turkey, roasted or broiled, including on sandwiches Chicken stew, chicken casserole, or stir-fry
8	Eggs	Eggs Egg substitutes, Egg Beaters
9	Butter	Butter on bread, potatoes, or vegetables
10	Margarine	Margarine on bread, potatoes, or vegetables
11	Yogurt	Yogurt, frozen yogurt (regular or low-fat)
12	Milk with lower fat content	2% milk (or chocolate 2% milk), not including on cereal Skim milk, 1% milk, not including on cereal Soy milk, Vita-Soy, Take Care soy drink
13	Whole milk	Whole milk (or whole chocolate milk), not including on cereal
14	Dairy products	Cheese dishes without tomato sauce, like macaroni and cheese Ice cream, regular or low-fat Cottage cheese Other cheeses and cheese spreads (regular or low-fat)
15	Beverage condiments	Cream, half and half or nondairy creamer in coffee or tea Milk in coffee or tea Sugar or honey in coffee or tea or on cereal
16	Liquor	Liquor or mixed drinks
17	Wine	Wine or wine coolers
18	Beer	Beer
19	Tea	Black tea, English tea, Chinese tea Green tea Chinese herbs made into or added to a soup or tea
20	Coffee	Coffee, not decaffeinated
21	Fruits	Apples and applesauce Bananas Peaches, apricots, canned or dried Peaches, apricots, fresh, in season Cantaloupe, in season Watermelon, in season Strawberries, other berries, in season Oranges or grapefruit, in season, not including juice Prunes, or prune juice Mangoes or papayas, fresh, in season
22	Fruit juices	Orange juice or grapefruit juice Apple juice or grape juice
23	Cruciferous vegetables	Broccoli Cauliflower or brussels sprouts Coleslaw, cabbage
24	Dark-yellow vegetables	Carrots, or mixed vegetables containing carrots Sweet potatoes, yams
25	Tomatoes	Tomatoes, tomato juice
26	Green leafy vegetables	Spinach, cooked or raw Mustard greens, turnip greens, collards, kale Green salad

27	Legumes	String beans, green beans Peas Beans such as baked beans, kidney beans, or in chili or bean burritos, not including soup Tofu, bean curd Alfalfa sprouts, including on sandwiches Regular bean sprouts Meat substitutes made from soy, like “soy burger”
28	Corn and corn products	Corn Cornbread, corn muffins, corn tortillas
29	Potatoes	White potatoes not fried, including boiled, baked, mashed & in potato salad
30	French fries	French fries and fried potatoes
31	Fiber cereals	Fiber cereals like raisin bran, granola or shredded wheat
32	Other cold cereals	Other cold cereals like corn flakes or cheerios Milk on cereal
33	Cooked cereal	Cooked cereals like oatmeal, oat bran or grits
34	Refined grains	Bagels, English muffins, hamburger buns Biscuits, muffins, including fast food Pancakes or waffles Spaghetti, lasagna, other pasta with tomato sauce Pasta salad, other pasta without tomato sauce Bread, including white bread, French, or whole wheat
35	Rice	Rice, or dishes made with rice
36	Pizza	Pizza, including carry-out
37	Snacks	Salty snacks, like potato chips, corn chips, popcorn, crackers Snacks like nachos with cheese, potato skins with topping
38	Peanuts/peanut butter	Peanuts, peanut butter
39	Sugar-sweetened beverages	Kool-Aid, Hi-C, or other drinks with added vitamin C Snapple, Calistoga, sweetened bottled waters or iced teas Regular cola soft drinks (not diet or ginger-ale type)
40	Artificially sweetened beverages	Diet cola soft drinks (not ginger-ale type)
41	Nutrition shakes	Instant breakfast milkshakes, diet shakes, or liquid supplements
42	Salad dressings	Salad dressing & mayonnaise, regular or low-fat
43	Soup	Vegetable soups with carrots or tomatoes, such as vegetable beef or tomato soup Other soups, like chicken noodle, mushroom, cup-a-soup, ramen Miso soup Lentil, pea and bean soups
44	Sweets and desserts	Doughnuts, pastry Pumpkin pie, sweet potato pie Other pies, including in restaurants Chocolate candy, candy bars Breakfast bars, granola bars, power bars Cookies or cake, regular or low-fat
45	Condiments	Salsa, ketchup, taco sauce Soy sauce, in cooking or added at the table
46	Mixed dishes	Beef or vegetable stew or pot pie with carrots and other vegetables

Supplemental Table 3.2 Characteristics of the study population by quartiles of dietary pattern factor scores from multiple statistical methods among participants of the Study of Women's Health Across the Nation[a]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*[b]
PCA-Western					
n	311	312	312	311	
Age at baseline, year, mean (SD)	46.3 (2.7)	46.2 (2.6)	46.3 (2.6)	46.4 (2.6)	0.81
Age at the carotid scan, year, mean (SD)	60.24 (2.8)	60.11 (2.7)	60.1 (2.6)	60.23 (2.7)	0.90
Race and ethnicity					< 0.001
African American, %	20.3	25.0	27.9	41.2	
Hispanic, %	6.4	6.4	4.5	6.4	
Chinese, %	27.0	10.6	6.4	6.8	
Non-Hispanic white, %	46.3	58.0	61.2	45.7	
Education level					0.0015
High school or less, %	19.9	18.9	23.4	21.9	
Some college, %	22.5	30.1	32.1	36.3	
College degree/post-college, %	57.6	51.0	44.6	41.8	
Somewhat/very hard to pay for basics, %	31.2	35.6	31.7	30.6	0.53
Self-rated overall health					0.63
Excellent/very good, %	64.0	61.9	66.7	61.4	
Good, %	25.4	29.5	26.0	28.6	
Fair/poor, %	10.6	8.7	7.4	10.0	
Depressive symptoms[c], %	19.3	23.7	25.6	21.5	0.26
Smoking status					0.10
Never, %	67.5	59.9	58.0	63.3	
Past, %	23.5	28.5	28.2	22.5	
Current, %	9.0	11.5	13.8	14.2	
Non-occupational physical activity[d], mean (SD)	8.0 (1.9)	7.7 (1.8)	7.7 (1.7)	7.6 (1.7)	0.024
Menopausal status					0.86
Early perimenopausal, %	42.4	45.2	44.2	45.7	
Premenopausal, %	57.6	54.8	55.8	54.3	
Hormone therapy use[e], %	39.6	48.1	44.9	40.2	0.10
Abdominal obesity, %	42.8	34.0	39.7	39.6	0.15
Elevated blood pressure, %	22.5	22.4	26.9	34.1	0.0024
Elevated fasting glucose, %	22.5	19.6	20.2	23.5	0.59
Elevated triglycerides, %	18.7	12.5	20.2	18.3	0.060
Reduced HDL cholesterol, %	32.8	32.4	34.9	33.1	0.91
CCA-IMT, mm[f], mean (SD)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.16
CCA-AD, mm[f], mean (SD)	7.2 (0.7)	7.2 (0.7)	7.2 (0.6)	7.2 (0.7)	0.58
High carotid plaque index[f, g], %	28.9	24.7	25.3	22.2	0.28
Number of missing dietary measurements					0.035
0, %	66.2	68.0	65.06	55.3	
1, %	23.8	21.5	24.68	31.8	
2, %	10.0	10.6	10.26	12.9	
PCA-Prudent					
n	311	312	312	311	
Age at baseline, year, mean (SD)	46.1 (2.5)	46.0 (2.7)	46.5 (2.6)	46.7 (2.7)	0.0023
Age at the carotid scan, year, mean (SD)	59.9 (2.6)	60.0 (2.7)	60.4 (2.7)	60.5 (2.8)	0.010
Race and ethnicity					< 0.001
African American, %	42.8	27.2	20.5	23.8	
Hispanic, %	11.9	8.3	2.6	1.0	
Chinese, %	5.1	12.8	17.0	15.8	
Non-Hispanic white, %	40.2	51.6	59.9	59.5	
Education level					< 0.001
High school or less, %	33.4	22.4	18.9	9.3	
Some college, %	35.1	31.1	28.2	26.7	
College degree/post-college, %	31.5	46.5	52.9	64.0	
Somewhat/very hard to pay for basics, %	42.8	28.5	31.7	26.1	< 0.001
Self-rated overall health					< 0.001
Excellent/very good, %	50.5	64.4	68.0	71.1	

Good, %	37.6	26.9	25.0	19.9	
Fair/poor, %	11.9	8.7	7.1	9.0	
Depressive symptoms[c], %	33.4	20.2	18.6	18.0	< 0.001
Smoking status					< 0.001
Never, %	56.3	62.2	66.4	64.0	
Past, %	21.5	28.9	23.1	29.3	
Current, %	22.2	9.0	10.6	6.8	
Non-occupational physical activity[d], mean (SD)	7.1 (1.7)	7.7 (1.7)	7.8 (1.8)	8.4 (1.8)	< 0.001
Menopausal status					0.23
Early perimenopausal, %	49.2	41.4	43.3	43.7	
Premenopausal, %	50.8	58.7	56.7	56.3	
Hormone therapy use[e], %	37.9	43.0	42.6	49.2	0.044
Abdominal obesity, %	44.1	39.7	38.1	34.1	0.083
Elevated blood pressure, %	29.6	25.3	24.4	26.7	0.48
Elevated fasting glucose, %	22.8	28.5	17.3	17.0	< 0.001
Elevated triglycerides, %	19.0	17.6	19.6	13.5	0.18
Reduced HDL cholesterol, %	36.7	35.6	31.1	29.9	0.20
CCA-IMT, mm[f], mean (SD)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.0036
CCA-AD, mm[f], mean (SD)	7.3 (0.6)	7.2 (0.7)	7.2 (0.6)	7.1 (0.7)	0.069
High carotid plaque index[f, g], %	26.1	27.9	23.7	23.5	0.54
Number of missing dietary measurements					< 0.001
0, %	50.5	59.6	71.8	72.7	
1, %	30.2	29.8	20.8	20.9	
2, %	19.3	10.6	7.4	6.4	
RRR-Western					
n	253	254	254	254	
Age at baseline, year, mean (SD)	46.7 (2.7)	46.4 (2.6)	45.9 (2.6)	46.4 (2.7)	0.0056
Age at the carotid scan, year, mean (SD)	60.6 (2.7)	60.2 (2.6)	59.7 (2.6)	60.1 (2.8)	0.0016
Race and ethnicity					< 0.001
African American, %	11.1	19.3	37.0	50.8	
Hispanic, %	0.0	0.0	0.4	2.0	
Chinese, %	27.3	18.1	9.8	1.6	
Non-Hispanic white, %	61.7	62.6	52.8	45.7	
Education level					< 0.001
High school or less, %	13.4	15.4	17.7	26.4	
Some college, %	24.9	20.9	33.5	42.1	
College degree/post-college, %	61.7	63.8	48.8	31.5	
Somewhat/very hard to pay for basics, %	24.5	26.8	34.3	34.3	0.026
Self-rated overall health					< 0.001
Excellent/very good, %	75.9	74.8	61.8	49.6	
Good, %	17.4	19.3	32.3	38.2	
Fair/poor, %	6.7	5.9	5.9	12.2	
Depressive symptoms[c], %	17.8	17.7	22.4	27.6	0.019
Smoking status					< 0.001
Never, %	63.6	70.9	62.2	53.5	
Past, %	28.9	24.4	26.0	24.8	
Current, %	7.5	4.7	11.8	21.7	
Non-occupational physical activity[d], mean (SD)	8.2 (1.8)	8.1 (1.8)	7.7 (1.8)	7.3 (1.6)	< 0.001
Menopausal status					0.12
Early perimenopausal, %	39.9	42.9	46.1	50.0	
Premenopausal, %	60.1	57.1	53.9	50.0	
Hormone therapy use[e], %	48.2	43.7	44.1	40.9	0.43
Abdominal obesity, %	23.7	31.5	48.8	51.6	< 0.001
Elevated blood pressure, %	20.2	21.7	25.6	37.4	< 0.001
Elevated fasting glucose, %	12.7	18.9	20.1	29.9	< 0.001
Elevated triglycerides, %	13.0	13.0	18.9	21.7	0.016
Reduced HDL cholesterol, %	24.1	30.3	36.2	37.8	0.0036
CCA-IMT, mm[f], mean (SD)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	1.0 (0.1)	< 0.001
CCA-AD, mm[f], mean (SD)	7.1 (0.6)	7.1 (0.6)	7.2 (0.6)	7.3 (0.7)	< 0.001

High carotid plaque index[f, g], %	26.1	20.5	25.6	32.7	0.020
Number of missing dietary measurements					< 0.001
0, %	79.1	78.7	72.8	63.8	
1, %	18.6	18.1	20.9	28.0	
2, %	2.4	3.2	6.3	8.3	
PLS-Western					
n	253	254	254	254	
Age at baseline, year, mean (SD)	46.8 (2.8)	46.2 (2.6)	46.0 (2.6)	46.3 (2.6)	0.0071
Age at the carotid scan, year, mean (SD)	60.7 (2.8)	60.1 (2.6)	59.8 (2.6)	60.1 (2.7)	0.0017
Race and ethnicity					< 0.001
African American, %	11.5	16.1	33.9	56.7	
Hispanic, %	0.0	0.0	0.4	2.0	
Chinese, %	30.0	17.3	7.5	2.0	
Non-Hispanic white, %	58.5	66.5	58.3	39.4	
Education level					< 0.001
High school or less, %	13.4	13.4	17.3	28.7	
Some college, %	20.6	23.2	34.3	43.3	
College degree/post-college, %	66.0	63.4	48.4	28.0	
Somewhat/very hard to pay for basics, %	26.1	25.2	34.7	33.9	0.029
Self-rated overall health					< 0.001
Excellent/very good, %	74.3	71.3	62.2	54.3	
Good, %	15.8	25.2	31.1	35.0	
Fair/poor, %	9.9	3.5	6.7	10.6	
Depressive symptoms[c], %	16.6	19.3	21.7	28.0	0.014
Smoking status					< 0.001
Never, %	68.4	68.9	55.9	57.1	
Past, %	28.5	22.8	31.9	20.9	
Current, %	3.2	8.3	12.2	22.1	
Non-occupational physical activity[d], mean (SD)	8.3 (1.8)	8.1 (1.7)	7.6 (1.8)	7.3 (1.6)	< 0.001
Menopausal status					0.40
Early perimenopausal, %	40.3	44.9	46.9	46.9	
Premenopausal, %	59.7	55.1	53.2	53.2	
Hormone therapy use[e], %	47.8	44.9	43.7	40.6	0.42
Abdominal obesity, %	29.3	36.6	41.7	48.0	< 0.001
Elevated blood pressure, %	19.0	23.6	24.0	38.2	< 0.001
Elevated fasting glucose, %	13.8	17.7	24.0	26.0	0.0020
Elevated triglycerides, %	13.4	17.7	15.4	20.1	0.21
Reduced HDL cholesterol, %	22.9	33.9	37.4	34.3	0.0030
CCA-IMT, mm[f], mean (SD)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	1.0 (0.2)	< 0.001
CCA-AD, mm[f], mean (SD)	7.1 (0.6)	7.1 (0.6)	7.2 (0.6)	7.3 (0.7)	< 0.001
High carotid plaque index[f, g], %	22.1	27.2	27.2	28.4	0.39
Number of missing dietary measurements					< 0.001
0, %	80.6	79.9	72.4	61.4	
1, %	16.6	17.7	23.6	27.6	
2, %	2.8	2.4	3.9	11.0	

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; HDL, high-density lipoprotein; IMT, intima-media thickness; PCA, principal component analysis; PLS, partial least squares regression; RRR, reduced rank regression; SD, standard deviation.

[a] Values are means (standard deviations) for continuous variables and percentages for categorical variables. Values of polytomous variables may not sum to 100% due to rounding. The variables are the baseline (1996-1997) measures unless specified otherwise.

[b] Computed by analysis of variance for continuous covariates and chi-square tests for binary/categorical covariates.

[c] Defined as the Center for Epidemiologic Studies Depression Scale ≥ 16.

[d] Assessed on five-point Likert and ordinal quantitative scales with total scores ranging from 3 to 15, with higher values indicating more frequent engagement in non-occupational physical activity.

[e] Defined as reported use of hormone therapy at any time from baseline to the visit of the carotid scan.

[f] Measured at Visit 12 (2009-2011) or Visit 13 (2011-2013).

[g] Carotid plaque index ≥ 2.

Supplemental Table 3.3 Quartiles of dietary pattern factor scores derived by multiple statistical methods and intima-media thickness of the common carotid artery among participants of the Study of Women's Health Across the Nation under various sensitivity analyses[a]

		Quartile 1		Quartile 2		Quartile 3		Quartile 4		*P*-trend[b]
	n	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI	
CCA-IMT, mm										
PCA-Western										
Main analyses[c]	1,246	0.923	0.900, 0.946	0.937	0.918, 0.956	0.957[i]	0.939, 0.976	0.964[i]	0.944, 0.985	0.013
Sensitivity 1 (Non-response weights)[d]	1,246	0.921	0.865, 0.976	0.937	0.890, 0.983	0.958[i]	0.919, 0.996	0.964[h]	0.934, 0.995	0.026
Sensitivity 2 (First two FFQs)[e]	1,004	0.927	0.903, 0.951	0.945	0.924, 0.966	0.969[i]	0.949, 0.990	0.971[i]	0.949, 0.993	0.021
Sensitivity 3 (Separately derived)[f]	1,004	0.937	0.913, 0.961	0.943	0.922, 0.964	0.961	0.941, 0.982	0.969	0.947, 0.991	0.052
Sensitivity 4 (MSM)[g]	861	0.936	0.907, 0.965	0.942	0.917, 0.967	0.962	0.939, 0.984	0.965	0.941, 0.990	0.12
PCA-Prudent										
Main analyses[c]	1,246	0.951	0.933, 0.969	0.955	0.937, 0.974	0.933	0.914, 0.952	0.945	0.926, 0.964	0.38
Sensitivity 1 (Non-response weights)[d]	1,246	0.950	0.920, 0.981	0.954	0.925, 0.983	0.933	0.905, 0.960	0.946	0.919, 0.972	0.49
Sensitivity 2 (First two FFQs)[e]	1,004	0.959	0.939, 0.979	0.957	0.937, 0.978	0.940	0.919, 0.961	0.955	0.934, 0.977	0.67
Sensitivity 3 (Separately derived)[f]	1,004	0.952	0.932, 0.971	0.963	0.943, 0.983	0.944	0.924, 0.965	0.956	0.935, 0.977	0.96
Sensitivity 4 (MSM)[g]	861	0.948	0.926, 0.970	0.967	0.944, 0.989	0.947	0.925, 0.969	0.954	0.932, 0.977	0.99
RRR-Western										
Main analyses[c]	1,015	0.934	0.909, 0.958	0.937	0.913, 0.961	0.950	0.927, 0.973	0.966[i]	0.945, 0.988	0.0058
Sensitivity 1 (Non-response weights)[d]	1,015	0.931	0.898, 0.963	0.935	0.901, 0.969	0.949	0.915, 0.984	0.967[i]	0.935, 0.999	0.0033
Sensitivity 2 (First two FFQs)[e]	875	0.937	0.913, 0.961	0.949	0.926, 0.972	0.961	0.939, 0.984	0.970[h]	0.949, 0.992	0.0086
Sensitivity 3 (Separately derived)[f]	875	0.937	0.913, 0.962	0.943	0.920, 0.966	0.961	0.939, 0.983	0.972[i]	0.951, 0.993	0.0048
Sensitivity 4 (MSM)[g]	775	0.933	0.908, 0.957	0.934	0.910, 0.959	0.959	0.935, 0.983	0.972[i]	0.949, 0.995	0.0011
PLS-Western										
Main analyses[c]	1,015	0.924	0.899, 0.948	0.942	0.918, 0.966	0.943	0.919, 0.966	0.973[j]	0.951, 0.994	< 0.001
Sensitivity 1 (Non-response weights)[d]	1,015	0.920	0.884, 0.957	0.939	0.901, 0.977	0.942	0.903, 0.981	0.974[j]	0.944, 1.004	< 0.001
Sensitivity 2 (First two FFQs)[e]	875	0.942	0.918, 0.965	0.954	0.931, 0.977	0.952	0.930, 0.974	0.974[h]	0.952, 0.995	0.026
Sensitivity 3 (Separately derived)[f]	875	0.940	0.917, 0.964	0.948	0.925, 0.972	0.959	0.937, 0.981	0.972[h]	0.950, 0.993	0.014
Sensitivity 4 (MSM)[g]	775	0.935	0.911, 0.960	0.947	0.923, 0.971	0.953	0.928, 0.978	0.973[i]	0.949, 0.997	0.0081

Abbreviations: CCA, common carotid artery; CI, confidence interval; FFQ, food frequency questionnaire; IMT, intima-media thickness; LS-mean, least squares mean; MSM, marginal structural model; PCA, principal component analysis; PLS, partial least squares regression; RRR, reduced rank regression.

[a] Values are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). The outcomes were measured at Visit 12 (2009-2011) or Visit 13 (2011-2013). All models were adjusted for age at the carotid scan (continuous), race/ethnicity (non-Hispanic white or not), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), smoking status (ever or never), non-occupational physical activity level (continuous), total energy intake (continuous; average of available visits), menopausal status (premenopausal or early perimenopausal), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced high-density lipoprotein cholesterol. The baseline covariates were used except age, total energy intake, and hormone therapy use.

[b] Computed by assigning the median factor score of each quartile to participants in the corresponding quartile as a continuous variable.

[c] Main analyses: The dietary patterns factor scores were derived using the food groups averaged across available visits of baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007).

[d] Sensitivity analysis 1: Inverse probability weighting to incorporate a non-response weight for each participant based on her baseline predictors of attrition.

[e] Sensitivity analysis 2: The dietary patterns factor scores were derived using the average food groups of baseline and Visit 5, restricting to women with dietary data from both time points.
[f] Sensitivity analysis 3: In addition to sensitivity analysis 2, the dietary patterns factor scores were derived separately for baseline and Visit 5 using the food groups at each visit, then the average factor scores were calculated and divided into quartiles.
[g] Sensitivity analysis 4: In addition to sensitivity analysis 3, also adjusted for abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced high-density lipoprotein cholesterol, all at Visit 3 (1999-2001), using inverse-probability-of-treatment weighting and marginal structural models.
[h] $P < 0.05$ (compared to Quartile 1).
[i] $P < 0.01$ (compared to Quartile 1).
[j] $P < 0.001$ (compared to Quartile 1).

Supplemental Table 3.4 Quartiles of dietary pattern factor scores derived by multiple statistical methods and adventitial diameter of the common carotid artery among participants of the Study of Women's Health Across the Nation under various sensitivity analyses[a]

		Quartile 1		Quartile 2		Quartile 3		Quartile 4		*P*-trend[b]
	n	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI	
CCA-AD, mm										
PCA-Western										
Main analyses[c]	1,246	7.30	7.19, 7.41	7.34	7.25, 7.43	7.39	7.31, 7.48	7.36	7.26, 7.46	0.65
Sensitivity 1 (Non-response weights)[d]	1,246	7.29	7.03, 7.56	7.34	7.12, 7.55	7.40	7.21, 7.58	7.37	7.21, 7.52	0.53
Sensitivity 2 (First two FFQs)[e]	1,004	7.30	7.19, 7.41	7.35	7.25, 7.45	7.34	7.24, 7.44	7.41	7.30, 7.51	0.20
Sensitivity 3 (Separately derived)[f]	1,004	7.31	7.20, 7.43	7.33	7.23, 7.43	7.34	7.24, 7.44	7.40	7.30, 7.51	0.21
Sensitivity 4 (MSM)[g]	861	7.34	7.21, 7.47	7.31	7.20, 7.43	7.35	7.25, 7.46	7.38	7.26, 7.50	0.46
PCA-Prudent										
Main analyses[c]	1,246	7.35	7.26, 7.43	7.39	7.30, 7.48	7.34	7.24, 7.43	7.33	7.24, 7.42	0.51
Sensitivity 1 (Non-response weights)[d]	1,246	7.34	7.19, 7.49	7.39	7.24, 7.53	7.34	7.20, 7.47	7.34	7.20, 7.47	0.64
Sensitivity 2 (First two FFQs)[e]	1,004	7.34	7.24, 7.43	7.36	7.27, 7.46	7.36	7.26, 7.46	7.36	7.26, 7.46	0.79
Sensitivity 3 (Separately derived)[f]	1,004	7.32	7.23, 7.41	7.38	7.29, 7.48	7.37	7.27, 7.47	7.34	7.24, 7.44	0.92
Sensitivity 4 (MSM)[g]	861	7.31	7.20, 7.42	7.40	7.30, 7.51	7.37	7.26, 7.48	7.34	7.23, 7.44	0.99
RRR-Western										
Main analyses[c]	1,015	7.33	7.22, 7.45	7.39	7.27, 7.50	7.36	7.25, 7.47	7.37	7.27, 7.48	0.56
Sensitivity 1 (Non-response weights)[d]	1,015	7.33	7.17, 7.49	7.38	7.21, 7.55	7.35	7.17, 7.52	7.36	7.20, 7.53	0.66
Sensitivity 2 (First two FFQs)[e]	875	7.35	7.23, 7.46	7.36	7.25, 7.47	7.44	7.33, 7.55	7.37	7.27, 7.47	0.59
Sensitivity 3 (Separately derived)[f]	875	7.35	7.24, 7.47	7.34	7.23, 7.45	7.47	7.37, 7.57	7.35	7.25, 7.45	0.68
Sensitivity 4 (MSM)[g]	775	7.34	7.22, 7.46	7.32	7.21, 7.44	7.48[h]	7.37, 7.60	7.36	7.25, 7.47	0.45
PLS-Western										
Main analyses[c]	1,015	7.31	7.19, 7.42	7.34	7.22, 7.46	7.33	7.22, 7.44	7.42	7.32, 7.52	0.071
Sensitivity 1 (Non-response weights)[d]	1,015	7.31	7.13, 7.49	7.33	7.15, 7.51	7.31	7.13, 7.49	7.42	7.26, 7.57	0.11
Sensitivity 2 (First two FFQs)[e]	875	7.32	7.21, 7.43	7.40	7.28, 7.51	7.38	7.27, 7.48	7.41	7.31, 7.51	0.20
Sensitivity 3 (Separately derived)[f]	875	7.32	7.20, 7.43	7.37	7.26, 7.48	7.39	7.29, 7.49	7.42	7.32, 7.52	0.097
Sensitivity 4 (MSM)[g]	775	7.31	7.19, 7.42	7.37	7.25, 7.49	7.38	7.27, 7.49	7.44[h]	7.32, 7.55	0.052

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CI, confidence interval; FFQ, food frequency questionnaire; LS-mean, least squares mean; MSM, marginal structural model; PCA, principal component analysis; PLS, partial least squares regression; RRR, reduced rank regression.

[a] Values are least squares means (95% CIs) from linear models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). The outcomes were measured at Visit 12 (2009-2011) or Visit 13 (2011-2013). All models were adjusted for age at the carotid scan (continuous), race/ethnicity (non-Hispanic white or not), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), smoking status (ever or never), non-occupational physical activity level (continuous), total energy intake (continuous; average of available visits), menopausal status (premenopausal or early perimenopausal), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced high-density lipoprotein cholesterol. The baseline covariates were used except age, total energy intake, and hormone therapy use.

[b] Computed by assigning the median factor score of each quartile to participants in the corresponding quartile as a continuous variable.

[c] Main analyses: The dietary patterns factor scores were derived using the food groups averaged across available visits of baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007).

[d] Sensitivity analysis 1: Inverse probability weighting to incorporate a non-response weight for each participant based on her baseline predictors of attrition.

[e] Sensitivity analysis 2: The dietary patterns factor scores were derived using the average food groups of baseline and Visit 5, restricting to women with dietary data from both time points.
[f] Sensitivity analysis 3: In addition to sensitivity analysis 2, the dietary patterns factor scores were derived separately for baseline and Visit 5 using the food groups at each visit, then the average factor scores were calculated and divided into quartiles.
[g] Sensitivity analysis 4: In addition to sensitivity analysis 3, also adjusted for abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced high-density lipoprotein cholesterol, all at Visit 3 (1999-2001), using inverse-probability-of-treatment weighting and marginal structural models.
[h] $P < 0.05$ (compared to Quartile 1).

Supplemental Table 3.5 Quartiles of dietary pattern factor scores derived by multiple statistical methods and carotid plaque among participants of the Study of Women's Health Across the Nation under various sensitivity analyses[a]

		Quartile 1	Quartile 2		Quartile 3		Quartile 4		*P*-trend[b]
	n	RR	RR	95% CI	RR	95% CI	RR	95% CI	
Carotid plaque									
PCA-Western									
Main analyses[c]	1,246	1.00	0.87	0.65, 1.15	0.85	0.61, 1.17	0.79	0.53, 1.17	0.35
Sensitivity 1 (Non-response weights)[d]	1,246	1.00	0.87	0.66, 1.16	0.84	0.61, 1.17	0.79	0.53, 1.18	0.36
Sensitivity 2 (First two FFQs)[e]	1,004	1.00	0.75	0.55, 1.02	0.75	0.54, 1.04	0.69	0.46, 1.05	0.22
Sensitivity 3 (Separately derived)[f]	1,004	1.00	0.74	0.55, 1.01	0.83	0.59, 1.16	0.70	0.47, 1.06	0.22
Sensitivity 4 (MSM)[g]	861	1.00	0.67[h]	0.48, 0.94	0.75	0.52, 1.07	0.64[h]	0.41, 0.99	0.18
PCA-Prudent									
Main analyses[c]	1,246	1.00	1.14	0.88, 1.47	1.02	0.77, 1.35	1.09	0.82, 1.50	0.74
Sensitivity 1 (Non-response weights)[d]	1,246	1.00	1.11	0.86, 1.43	1.01	0.76, 1.34	1.10	0.82, 1.47	0.69
Sensitivity 2 (First two FFQs)[e]	1,004	1.00	1.19	0.89, 1.61	1.43[h]	1.07, 1.91	1.05	0.75, 1.47	0.71
Sensitivity 3 (Separately derived)[f]	1,004	1.00	1.33	0.99, 1.79	1.43[h]	1.06, 1.94	1.15	0.82, 1.60	0.53
Sensitivity 4 (MSM)[g]	861	1.00	1.37	0.99, 1.91	1.55[i]	1.12, 2.15	1.11	0.78, 1.60	0.72
RRR-Western									
Main analyses[c]	1,015	1.00	0.77	0.56, 1.05	0.86	0.63, 1.18	1.01	0.75, 1.35	0.79
Sensitivity 1 (Non-response weights)[d]	1,015	1.00	0.75	0.55, 1.03	0.82	0.60, 1.13	1.02	0.76, 1.37	0.71
Sensitivity 2 (First two FFQs)[e]	875	1.00	0.87	0.64, 1.20	0.71	0.50, 1.01	0.96	0.70, 1.30	0.77
Sensitivity 3 (Separately derived)[f]	875	1.00	1.04	0.74, 1.45	0.87	0.61, 1.23	1.17	0.85, 1.61	0.42
Sensitivity 4 (MSM)[g]	775	1.00	1.10	0.77, 1.56	0.91	0.64, 1.30	1.17	0.84, 1.64	0.46
PLS-Western									
Main analyses[c]	1,015	1.00	1.17	0.86, 1.58	1.06	0.78, 1.46	1.10	0.80, 1.51	0.66
Sensitivity 1 (Non-response weights)[d]	1,015	1.00	1.18	0.87, 1.61	1.04	0.75, 1.43	1.13	0.82, 1.56	0.59
Sensitivity 2 (First two FFQs)[e]	875	1.00	1.00	0.73, 1.39	0.86	0.62, 1.20	0.97	0.70, 1.36	0.73
Sensitivity 3 (Separately derived)[f]	875	1.00	1.06	0.77, 1.47	0.86	0.61, 1.20	1.00	0.71, 1.39	0.76
Sensitivity 4 (MSM)[g]	775	1.00	1.05	0.75, 1.47	0.88	0.63, 1.25	0.98	0.69, 1.40	0.74

Abbreviations: CI, confidence interval; FFQ, food frequency questionnaire; MSM, marginal structural model; PCA, principal component analysis; PLS, partial least squares regression; RR, risk ratio; RRR, reduced rank regression.

[a] Values are risk ratios (95% CIs) of high carotid plaque index (≥ 2) from log-binomial models. Modified Poisson models with robust variance estimation were used to achieve model convergence. The outcomes were measured at Visit 12 (2009-2011) or Visit 13 (2011-2013). All models were adjusted for age at the carotid scan (continuous), race/ethnicity (non-Hispanic white or not), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), smoking status (ever or never), non-occupational physical activity level (continuous), total energy intake (continuous; average of available visits), menopausal status (premenopausal or early perimenopausal), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced high-density lipoprotein cholesterol. The baseline covariates were used except age, total energy intake, and hormone therapy use.

[b] Computed by assigning the median factor score of each quartile to participants in the corresponding quartile as a continuous variable.

[c] Main analyses: The dietary patterns factor scores were derived using the food groups averaged across available visits of baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007).

[d] Sensitivity analysis 1: Inverse probability weighting to incorporate a non-response weight for each participant based on her baseline predictors of attrition.

[e] Sensitivity analysis 2: The dietary patterns factor scores were derived using the average food groups of baseline and Visit 5, restricting to women with dietary data from both time points.
[f] Sensitivity analysis 3: In addition to sensitivity analysis 2, the dietary patterns factor scores were derived separately for baseline and Visit 5 using the food groups at each visit, then the average factor scores were calculated and divided into quartiles.
[g] Sensitivity analysis 4: In addition to sensitivity analysis 3, also adjusted for abdominal obesity, elevated blood pressure, elevated fasting glucose, elevated triglycerides, and reduced high-density lipoprotein cholesterol, all at Visit 3 (1999-2001), using inverse-probability-of-treatment weighting and marginal structural models.
[h] $P < 0.05$ (compared to Quartile 1).
[i] $P < 0.01$ (compared to Quartile 1).

Chapter 4. Prospective Associations Between A Healthy Lifestyle During the Midlife and Subclinical Carotid Atherosclerosis

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4.1 Abstract

Background: Measures of subclinical atherosclerosis are predictors of future cardiovascular outcomes as well as of physical and cognitive functioning. The menopausal transition is associated with accelerated progression of atherosclerosis in women. The prospective association between a healthy lifestyle during the midlife and subclinical atherosclerosis is unclear.

Methods: Self-reported data on smoking, diet, and physical activity from 1,143 women in the Study of Women's Health Across the Nation were used to construct a 10-year average Healthy Lifestyle Score (HLS) during the midlife. Markers of subclinical atherosclerosis were measured 14 years after baseline and included common carotid artery intima-media thickness (CCA-IMT), adventitial diameter (CCA-AD), and carotid plaque. The associations of average HLS with CCA-IMT and CCA-AD were estimated using linear models; the association of average HLS with carotid plaque was estimated using cumulative logit models.

Results: Average HLS was associated with smaller CCA-IMT and CCA-AD in the fully adjusted models (*P*: 0.0031 and <0.001, respectively). Compared to participants in the lowest HLS level, those in the highest level had 0.024 mm smaller CCA-IMT (95% CI: -0.048, 0.000), which equals 17% of the standard deviation (SD) of CCA-IMT, and 0.16 mm smaller CCA-AD (95% CI: -0.27, -0.04), which equals 24% of the SD of CCA-AD. Among the three components of the HLS, abstinence from smoking had the strongest association with subclinical atherosclerosis.

Conclusions: Healthy lifestyle during the menopausal transition is associated with less subclinical atherosclerosis, highlighting the growing recognition that the midlife is a critical window for cardiovascular prevention in women.

4.2 Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States in women as well as in men.[1] Women experience a steeper increase in CVD risk during and after the menopausal transition relative to before menopause.[1-3] Menopause is also associated with several adverse changes of cardiovascular risk factors independently of chronological aging, such as increased levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and apolipoprotein B.[6] This suggests that the midlife may be an especially relevant period for cardiovascular risk assessment and prevention in women.

Subclinical atherosclerosis is closely related to the onset of clinically apparent CVD and to CVD mortality. The development of subclinical atherosclerosis typically precedes the occurrence of clinical CVD by years to decades.[9] Measures of subclinical atherosclerosis of the common carotid artery (CCA), such as intima-media thickness (IMT), adventitial diameter (AD), and carotid plaque, are clinically important predictors of future CVD events[12, 13, 15] and are useful in quantifying CVD risk in asymptomatic individuals without clinically diagnosed CVD.[12-14] In addition to being predictive of clinical outcomes, measures of subclinical carotid atherosclerosis are also associated with poorer physical and cognitive functioning in old age independent of clinical CVD.[21-23] The distribution and determinants of subclinical atherosclerosis differ substantially by age and sex.[25] It has been shown previously that the menopausal transition is associated with accelerated progression of subclinical carotid atherosclerosis.[11, 26]

Abstinence from smoking, adoption of a healthy diet, and engagement in regular physical activity are three well-known modifiable behavioral factors that are considered part of a heart-healthy lifestyle. Prior studies have found inverse associations between an overall healthy lifestyle and various CVD outcomes, including coronary heart disease,[80] myocardial infarction,[81]

and CVD-related mortality.[82] However, to the best of our knowledge, no study has examined the prospective association between the long-term lifestyle in midlife women and subclinical atherosclerosis. Because of the association of subclinical atherosclerosis with future clinical CVD and physical/cognitive functioning, as well as the accelerated progression of atherosclerosis during the menopausal transition, the potential effect of modifiable health behaviors on subclinical atherosclerosis in midlife women warrants further investigation, as the midlife may be a critical window of opportunity for prevention. A prior study shows that a lifestyle education program targeting diet and physical activity might be able to slow the menopause-related progression of atherosclerosis.[26] Therefore, we aimed to use data from the Study of Women's Health Across the Nation (SWAN) to create a composite Healthy Lifestyle Score (HLS) from three behavioral CVD risk factors that are largely modifiable (smoking, diet quality, and physical activity) and to evaluate the prospective association between the HLS during the midlife and measures of subclinical carotid atherosclerosis. We also aimed to explore the independent association between each component of the HLS and subclinical carotid atherosclerosis.

4.3 Methods

4.3.1 Study Design and Study Population

The Study of Women's Health Across the Nation (SWAN) is an ongoing, multicenter, multiethnic, prospective cohort study initiated in 1996 to study the natural history of the menopausal transition. Details of the SWAN protocol have been described previously.[87] Briefly, SWAN participants were recruited from seven sites across the United States: 1) Boston, Massachusetts; 2) Chicago, Illinois; 3) Southeastern Michigan; 4) Los Angeles, California; 5) Newark, New Jersey; 6) Pittsburgh, Pennsylvania; and 7) Oakland, California. Women who identified themselves as African American (Pittsburgh, Chicago, Detroit, and Boston), Chinese (Oakland), Japanese (Los Angeles), Hispanic (Newark), or non-Hispanic white (all sites) were enrolled. Baseline eligibility criteria included: age 42 to 52 years, having an intact uterus and at least one ovary, not being pregnant or lactating, not using oral contraceptives or hormone therapy in the past three months, and having at least one menstrual cycle in the past three months. The initial sample size at baseline was 3,302. Clinic assessments began in 1996 and the participants have been followed up for 15 approximately annual examinations through the most recent visit in 2015-2016. The SWAN protocols were approved by the Institutional Review Board at each site, and all participants provided written informed consent at each study visit.

Carotid ultrasound scans were conducted at six sites (except the Los Angeles site) at SWAN follow-up Visit 12 (2009-2011) or Visit 13 (2011-2013), with the vast majority of scans performed at Visit 12. Among the 2,806 women initially enrolled at these six sites, 1,990 (70.9%) participants attended Visit 12, of whom 1,592 (80.0%) had a carotid scan at either Visit 12 or Visit 13. Additionally, 14 women did not attend Visit 12, but attended and received their

carotid scan at Visit 13. Thus, a total of 1,606 women had a carotid scan at either Visit 12 or Visit 13. From these 1,606 participants, we further excluded women who did not have all three specific measures of carotid atherosclerosis (n = 54); who self-reported having heart disease (n = 51) or stroke (n = 9) at baseline or who developed heart disease (n = 39) or stroke (n = 36) during the follow-up; who reported too few (< 4) or too many (> 16) numbers of solid foods per day (n = 110), skipped more than 10 food items on the food frequency questionnaire (n = 3) or reported a total energy intake that was too low (< 2,092 kJ/day, i.e., 500 kcal/day) or too high (>20,920 kJ/day, i.e, 5,000 kcal/day) (n = 4); who had incomplete data on the three components of HLS for all visits (n = 21); and who had missing data for the major covariates (n = 136). After these exclusions, the final analytical sample consisted of 1,143 women (**Figure 4.1**); 98% (n = 1,121) of the retained participants received their carotid ultrasound scan at Visit 12.

Comparisons of baseline characteristics of women in the analytic sample (n = 1,143) with those excluded (n = 861) showed that the excluded participants were significantly ($P < 0.05$) more likely to be African American (42.6% of the excluded women vs. 26.2% of the retained women were African American) and early perimenopausal (48.7% vs. 43.7%), and were significantly more likely to report difficulty paying for basic necessities (48.0% vs. 31.0%) and depressive symptoms (28.2% vs. 21.8%), to have a higher body mass index (mean: 29.9 kg/m^2 vs. 27.9 kg/m^2), elevated blood pressure (37.0% vs. 26.2%), elevated fasting glucose (30.3% vs. 20.6%), and reduced high-density lipoprotein (HDL) cholesterol (41.0% vs. 32.6%). At baseline, the excluded participants were significantly less likely to have a college degree (39.5% of the excluded women vs. 48.9% of the retained women had a college degree), to be married or living as if married (58.3% vs. 70.3%), and to self-report having excellent/very good overall health (51.3% vs. 64.5%). Age at baseline, age at the carotid scan, hormone therapy use during the

follow-up, and baseline proportions of elevated serum triglycerides, elevated total cholesterol, and elevated LDL cholesterol did not differ significantly between the retained and the excluded participants.

4.3.2 Assessment of Exposures

In a prior study in SWAN, Sternfeld et al. created a healthy lifestyle index using smoking, physical activity, and diet quality (quantified by a healthy diet score).[117] Building on this prior work, we modified the original index by using the well-established Alternate Healthy Eating Index (AHEI) to quantify diet quality, while keeping the scoring methods of smoking and physical activity the same as in the original index. The AHEI is an *a priori* dietary index that quantifies the adherence of one's diet to certain dietary guidelines. The AHEI has repeatedly been shown to be predictive of the risk of chronic disease and can better account for the types of fiber, protein, and fats.[94, 118, 119]

Dietary data were collected at baseline (1996-1997), Visit 5 (2001-2003), and Visit 9 (2005-2007) using a modification of the 1995 version of the Block food frequency questionnaire (FFQ), which has previously been validated against dietary records[88] and 24-hour recalls.[89] Briefly, among women, the deattenuated correlation coefficients (i.e., after removing the random, within-person errors)[120] between the Block FFQ and 24-hour recalls for total energy intake, protein, carbohydrate, total fat, saturated fat, monounsaturated fat, and polyunsaturated fat were 0.45, 0.53, 0.66, 0.67, 0.65, 0.60, and 0.48, respectively.[89] The FFQ used in SWAN included 103 food items, including 83 solid food items and 20 beverage food items. The FFQ was administered by trained personnel. The participants were asked how often, on average, they

consumed each food of a standard portion size during the past year. Up to nine possible responses were available for each food item: <once/month or never, once/month, 2-3 times/month, once/week, twice/week, 3-4 times/week, 5-6 times/week, once/day, and ≥twice/day. We further transformed the responses into semi-continuous variables representing servings per day, with values 0.016, 0.03, 0.08, 0.14, 0.28, 0.5, 0.79, 1, and 2 for the nine responses, respectively. Intake of energy and nutrients was computed by multiplying the consumption frequency of each food by the corresponding nutrient content.

For each visit, we calculated the AHEI for each participant. The AHEI includes nine components of foods and nutrients intake (**Supplemental Table 4.1**). The scores of the nine components were summed to obtain the total AHEI score which ranged from 2.5 (worst diet) to 87.5 (best diet). We further collapsed each participant's AHEI score and gave a score of 2 when the participant's AHEI score was in the top tertile of the study population, a score of 1 when the AHEI score was in the middle tertile, and a score of 0 when the AHEI score was in the bottom tertile.

Current guidelines recommend that adults should pursue at least 150 minutes/week of moderate-intensity, or 75 minutes/week of vigorous-intensity, aerobic physical activity (or an equivalent combination of both) for substantial health benefits.[79] Physical activity was evaluated from the sports and exercise questions on the validated Kaiser Physical Activity Survey[121] to determine whether this recommendation was met.[117] We gave a score of 2 (fully meeting the recommendation) to those who played sports or exercised more than once a week, for at least 2 hours/week for at least nine months during the past year and with at least a moderate increase in heart rate and breathing. We gave a score of 1 (partially meeting the recommendation) to those who played sports or exercised more than once a month but no more than once a week, or to

those who played sports or exercised more than once a week but did not satisfy other criteria to qualify for a score of 2. We gave a score of 0 (not meeting the recommendation) to those who played sports or exercised no more than once a month. Data of physical activity at baseline, Visit 5 and Visit 9 were used (i.e., the same time points as the available dietary data).

Standardized questions from the American Thoracic Association[122] were used to collect information on smoking status. We gave a score of 2 to never smoking, a score of 1 to past smoking, and a score of 0 to current smoking. Data at baseline, Visit 5 and Visit 9 were used.

To calculate the Healthy Lifestyle Score (HLS), we computed the arithmetic sum (i.e., without weighting) of the scores for the individual components of smoking, physical activity, and diet quality to create visit-specific HLS, with a possible range of 0 to 6 for each visit (baseline, Visit 5, and Visit 9). The visit-specific scores were then averaged across all non-missing visits to create the average HLS. To uncover potential non-linear associations, we further divided the average HLS into four levels: 0 to 2, >2 to 3, >3 to 4, and >4 to 6, which approximated the quartiles of the HLS distribution.

4.3.3 Assessment of Outcomes

The details of the carotid ultrasound measurements have been described elsewhere.[90, 123] Briefly, at all SWAN sites except the Los Angeles site, centrally trained and certified sonographers obtained carotid ultrasound images at Visit 12 (and Visit 13 for a small group of participants), using a Terason t3000 Ultrasound System (Teratech Corp, Burlington, MA) equipped with a variable frequency (5-12 MHz) linear array transducer. Two digitized images were obtained of each of the left and right distal common carotid artery (CCA). From each of

these 4 images, using the AMS semi-automated edge detection software,[124] near and far wall intima-media thickness (IMT) measures of the CCA were obtained by electronically tracing the lumen-intima interface and the media-adventitia interface across a 1-cm segment proximal to the carotid bulb; one measurement was generated for each pixel over the area, for a total of approximately 140 measures for each segment. The average and maximal values for these measures were recorded for all four images, with the mean of the maximal readings of all four images used in the analyses. Adventitial diameter (AD) of the CCA was measured as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interface on the far wall at end-diastole across the same CCA segments used for IMT measurement. The mean value of the average readings was used in the analyses. Carotid scan images were read centrally at the SWAN Ultrasound Reading Center (University of Pittsburgh Ultrasound Research Laboratory). Sonographers at each site evaluated the presence and extent of plaque in each of 5 segments of the left and right carotid artery (distal and proximal common carotid artery, carotid bulb, and proximal internal and external carotid arteries). A plaque was defined as a distinct area protruding into the vessel lumen that was at least 50% thicker than the adjacent IMT. For each segment, the degree of the plaque was graded between 0 (no observable plaque) to 3 (plaque obstructing ≥ 50% of the luminal diameter of the vessel). The grades from all segments of the combined left and right carotid artery were summed to create the plaque index.[91] Technicians at the six study sites were trained by the University of Pittsburgh Ultrasound Research Laboratory and monitored during the study period for reliability. Reproducibility of IMT measures was good to excellent with an intraclass correlation coefficient between sonographers of ≥ 0.77, and between readers of > 0.90. The plaque index was similarly reliable with an intraclass correlation ranging from 0.86 to 0.93.[125]

The three outcomes of this study were the intima-media thickness of the common carotid artery (CCA-IMT), the adventitial diameter of the common carotid artery (CCA-AD), and the extent of carotid plaque (categorized by carotid plaque index). We treated CCA-IMT and CCA-AD as continuous outcome variables. We collapsed carotid plaque index into none (0), moderate (1), and high (≥ 2) and treated it as a categorical (ordinal) outcome variable.

4.3.4 Assessment of Covariates

Self-reported covariates at baseline included age (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), depressive symptoms (dichotomized by the Center for Epidemiologic Studies Depression Scale: ≥16 or <16),[92] and menopausal status based on self-reported menstrual bleeding patterns (dichotomized as premenopausal or early perimenopausal).[126] Self-reported use of hormone therapy during the follow-up was dichotomized as ever use or never use, with ever use defined as use of hormone therapy at any visit from baseline to the visit of the carotid scan. The presence of hot flash was self-reported at Visit 12. Weight and height were measured by trained interviewers using a calibrated balance beam scale and a stadiometer, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. Blood pressure was calculated as the average of two seated measurements using a standard mercury sphygmomanometer. Blood samples were taken to measure fasting glucose, total

cholesterol, serum triglycerides, LDL cholesterol, and HDL cholesterol. Based on harmonized guidelines,[96] elevated blood pressure was defined as systolic blood pressure ≥ 130 mm Hg, or diastolic blood pressure ≥ 85 mm Hg, or use of at least one antihypertensive medication. Elevated fasting glucose was defined as fasting glucose ≥ 100 mg/dL or use of at least one antidiabetic medication. Elevated serum triglycerides was defined as fasting serum triglycerides ≥ 150 mg/dL. Reduced HDL cholesterol was defined as serum HDL cholesterol < 50 mg/dL. Additionally, we considered total cholesterol ≥ 200 mg/dL as elevated total cholesterol, and LDL cholesterol ≥ 130 mg/dL as elevated LDL cholesterol.

4.3.5 Statistical Analysis

In the descriptive analysis, we computed the means and standard deviations for continuous covariates and percentages for categorical covariates for the entire study population as well as stratified by categories of average HLS, adjusting for baseline age. In the multivariate regression analysis, we estimated the association of the levels of the average HLS (0 to 2; >2 to 3; >3 to 4; and >4 to 6) with CCA-IMT and CCA-AD using linear models, and with carotid plaque (high vs. moderate vs. none) using cumulative logit models (i.e., ordinal logistic regression models). No transformations were performed on CCA-IMT or CCA-AD. Normality and homoscedasticity of the residuals were checked and satisfied in all linear models. No significant deviation from the proportional odds assumption was observed (*P* values for the score tests > 0.05) in the cumulative logit models.[127]

The selection of confounders was based on *a priori* knowledge of risk factors for subclinical atherosclerosis gained from the literature and supplemented by the empirical

exposure-covariate associations in the study population. We adjusted for baseline covariates, age at the carotid scan, use of hormone therapy from baseline to the visit of the carotid scan, self-reported hot flash at Visit 12, and the number of missing visits for the HLS. The baseline covariates included race/ethnicity, education level, financial strain, marital status, self-rated overall health, depressive symptoms, total energy intake, and menopausal status. Physiological risk factors, including body mass index, elevated blood pressure, elevated fasting glucose, serum triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, use of antilipidemic medications, and use of antihypertensive medications might be both confounders and potential mediators. Therefore, the values of these covariates were additionally adjusted for in separate models. *P* values were computed by using the HLS as a continuous variable in the models. We further explored the association of each component of the HLS (i.e., smoking, diet, and physical activity) with subclinical carotid atherosclerosis individually by adjusting for the other two components in the same model.

We additionally conducted a series of sensitivity analyses to ensure the robustness of the results. First, we generated a weighted HLS by using the percentage of the coefficient of each individual component to the sum of the coefficients in the models with all components included. To capture any potential U-shaped association between physical activity and atherosclerosis (possibly observable only in the presence of extreme physical exertion levels far above the recommendation),[128] we included both the linear and the quadratic terms of physical activity in the creation of the weighed HLS. Second, to assess selection bias due to the attrition and missing data, we used inverse probability weighting to develop a non-response weight for each retained participant based on the baseline predictors of attrition, including race/ethnicity, education level, financial strain, marital status, self-rated overall health, depressive symptoms, body mass index,

menopausal status, elevated blood pressure, elevated fasting glucose, and reduced HDL cholesterol. We repeated the analyses using the weights. Third, we repeated the analyses by adjusting for financial strain, marital status, self-rated overall health, and depressive symptoms at the visit of the carotid scan rather than at baseline. Fourth, we repeated the analysis by including only the women with HLS data from all three time points (baseline, Visit 5, and Visit 9). Fifth, we treated carotid plaque as a binary outcome (carotid plaque index ≥ 2 versus. < 2) instead of an ordinal outcome and used logistic regression models. Sixth, we repeated the analysis using the three visit-specific HLS values instead of the average HLS. All analyses were conducted using SAS (Version 9.4. SAS Institute Inc., Cary, NC) at a two-sided alpha level of 0.05. The first author had full access to all the primary data in the study and takes responsibility for the data analysis.

4.4 Results

Descriptive Analyses

Among the 1,143 participants in the analytical sample, those with a high average Healthy Lifestyle Score (HLS) were more likely to have a college degree, self-report their overall health to be excellent or very good, have a lower BMI at baseline, be premenopausal at baseline, and use hormone therapy during the follow-up. Participants with a high average HLS were less likely to be Hispanic or African American, experience difficulty paying for basics, be separated/widowed/divorced, experience depressive symptoms, and less likely to have elevated blood pressure, elevated fasting glucose, elevated serum triglycerides, elevated total cholesterol, reduced HDL cholesterol, and elevated LDL cholesterol at baseline (**Table 4.1**).

The HLS scores were relatively stable over time. The Spearman correlation coefficient for the HLS score was 0.66 between baseline and Visit 5, 0.70 between Visit 5 and Visit 9, and 0.66 between baseline and Visit 9. Smoking status did not appear to change considerably over time, as 93.4% of the study population did not report a change in smoking status (in terms of current, past, and never) from baseline to Visit 9. Over half (54.2%) of the study population reported a change in their physical activity status (in terms of fully meeting, partially meeting, and not meeting the recommendation) from baseline to Visit 9. The Alternate Healthy Eating Index (AHEI) scores were moderately stable over time. The Pearson correlation coefficient of the AHEI score was 0.63 between baseline and Visit 5, 0.69 between Visit 5 and Visit 9, and 0.62 between baseline and Visit 9. While 711 participants (62.2% of the study population) remained as never smokers at all three visits, only 204 (17.8% of the study population) consistently stayed in the top tertile of the AHEI scores, and only 82 (7.2% of the study population) self-reported physical activity status that consistently met the recommendation. Only

19 participants (1.7% of the study population) remained in the top category for all three components at all three visits.

HLS and Subclinical Carotid Atherosclerosis

The average HLS over 10 years of follow-up was inversely and statistically significantly associated with CCA-IMT and CCA-AD (**Figure 4.2** and **Table 4.2**). The inverse associations persisted even after adjusting extensively for confounders and physiological risk factors (*P*: 0.0031 and < 0.001 for CCA-IMT and CCA-AD, respectively). In the fully adjusted models, compared to participants in the lowest level of average HLS (i.e., 0 to 2), those in the highest level (i.e., >4 to 6) had a 0.024 mm smaller CCA-IMT (95% CI of beta coefficient: -0.048, 0.000), a difference that equals 17% of the standard deviation (SD) of CCA-IMT in the analytical sample; they also had a 0.16 mm smaller CCA-AD (95% CI of beta coefficient: -0.27, -0.04), a difference that equals 24% of the SD of CCA-AD. We also observed an inverse association between average HLS and carotid plaque after adjusting for major confounders (P = 0.024), although the association failed to retain its statistical significance after additionally adjusting for physiological risk factors (P = 0.25).

Components of HLS and Subclinical Carotid Atherosclerosis

We estimated the independent associations of each component of the HLS by adjusting for the other two components in the same model (**Tables 4.3-4.5**, for smoking, diet quality, and physical activity, respectively). Abstinence from smoking was strongly and inversely associated with all three measures of subclinical carotid atherosclerosis (P-trend < 0.01 for all three outcome measures). Compared to the participants who smoked at some point during the follow-

up, those who remained never smokers had a 0.047 mm smaller CCA-IMT (95% CI of beta coefficient: -0.070, -0.024; a 34% SD difference), a 0.24 mm smaller CCA-AD (95% CI of beta coefficient: -0.35, -0.13; a 36% SD difference), and 49% lower odds of having a higher carotid plaque index (OR = 0.51; 95% CI: 0.35, 0.73) (**Table 4.3**). We observed an inverse association between average AHEI score and CCA-AD after adjusting for major confounders and physiological risk factors (except BMI) (*P*-trend = 0.016). The association lost significance after further adjusting for BMI (*P*-trend = 0.11). We observed a marginally significant inverse association between average AHEI score and CCA-IMT (*P*-trend = 0.067), which also lost significance after further adjusting for baseline BMI (*P*-trend = 0.18). We found no independent association between average AHEI score and carotid plaque (**Table 4.4**). Long-term physical activity status was marginally significantly associated with CCA-IMT after adjusting for major confounders (*P*-trend = 0.059). The association lost its significance after additionally adjusting for the physiological risk factors. We found no independent association between long-term physical activity status and CCA-AD or carotid plaque (**Table 4.5**).

Sensitivity Analyses

Results from the sensitivity analyses were consistent with the primary analyses. The estimates were similar after using the weighted HLS and including a quadratic term for physical activity (**Supplemental Table 4.2**). The results did not change appreciably after accounting for missing data using the non-response weights, adjusting for certain covariates at the visit of the carotid scan (instead of at baseline), and treating carotid plaque as a binary outcome. The sensitivity analysis that focused on women with HLS data from all three time points showed stronger associations between average HLS and subclinical carotid atherosclerosis compared to

the primary analysis. The results were qualitatively similar when using the three visit-specific HLS values as the exposures. The HLS values at baseline and Visit 5 displayed weaker associations with the outcomes compared to the average HLS, whereas the estimates for the HLS at Visit 9 were similar to those for the average HLS (data not shown).

4.5 Discussion

This study evaluated the prospective associations between a composite midlife Healthy Lifestyle Score (HLS) and measures of subclinical carotid atherosclerosis in women. The HLS includes three health behaviors, smoking, diet quality, and physical activity, that are largely modifiable. We found that the level of average HLS during the midlife over 10 years of follow-up was significantly associated with smaller CCA-IMT and CCA-AD, approximately 14 years after baseline. Among the three individual components of the HLS, abstinence from smoking displayed the strongest inverse associations with subclinical carotid atherosclerosis.

Accumulating evidence from prior studies indicates that measures of subclinical carotid atherosclerosis are important predictors of future CVD events[12, 13, 15] and useful indicators of cardiovascular risk in apparently healthy individuals.[12-14] In a meta-analysis,[15] Lorenz et al. found that, after adjusting for age and sex, every one-SD increase in CCA-IMT was associated with a 26% higher risk of myocardial infarction and a 32% higher risk of stroke. Similarly, using data from the Atherosclerosis Risk in Communities Study, Eigenbrodt et al. reported that every one-SD increase in CCA-AD was associated with 18% higher hazard of incident cardiac events and 73% higher odds of prevalent myocardial infarction in women even after adjusting for CCA-IMT and other CVD risk factors.[16] Likewise, the presence of carotid plaque is associated with 83%, 210%, and 81%-196% higher hazard of myocardial infarction,[17] stroke,[18] and death from coronary heart disease,[19, 20] respectively, in women without known cardiovascular disease. Furthermore, the extent of subclinical carotid atherosclerosis is associated with poorer physical[21] and cognitive[21-23] functioning even among individuals without clinical CVD or after adjusting for the intervening clinical CVD outcomes in the analysis.

Smoking, unhealthy diet, and lack of physical activity are three well-known modifiable behavioral risk factors for CVD. In one of the earliest investigations into overall lifestyle factors and CVD, Stampfer et al. followed midlife women in the Nurses' Health Study (NHS) for 14 years for the occurrence of major coronary events.[80] They found that women who did not smoke, who engaged in moderate-to-vigorous physical activity for ≥ 30 minutes/day, and who had a healthy diet (scored in the upper 2 quintiles for a diet high in cereal fiber, marine n-3 fatty acids, folate, the ratio of polyunsaturated to saturated fatty acids, and low in *trans* fat and glycemic load) had 57% lower odds of a coronary event compared with all the other women who did not have this healthy lifestyle.[80] Qualitatively similar results indicating an inverse association between a healthy lifestyle and clinical CVD have been reported in other studies.[81, 82] Notably, a recent analysis used data from the NHS and reported that women with five low-risk lifestyle factors (never smoking, normal BMI, moderate-to-vigorous physical activity, moderate alcohol intake, and high diet quality) had 74% lower hazard of all-cause mortality and 82% lower hazard of CVD mortality, compared to those with zero low-risk factors.[83] The projected life expectancy at age 50 years comparing women with five low-risk factors with women with zero low-risk factors was 14 years (95% CI: 11.8, 16.2) longer, 30.8% of which was attributable to reduced CVD death.[83]

The potential effect of a healthy lifestyle on the extent of subclinical atherosclerosis has been evaluated in few prior studies.[116, 129, 130] Moreover, to the best of our knowledge, no study has examined the association between a composite healthy lifestyle index and subclinical carotid atherosclerosis in midlife women undergoing the menopausal transition. There are significant age and sex differences in the distribution and the determinants of subclinical atherosclerosis.[25] Independent of chronological aging, the menopausal transition represents a vulnerable window

of increased cardiovascular risk[1-3] as well as an accelerated progression of subclinical carotid atherosclerosis.[11, 26] As reported in a previous SWAN study, the progression rate of both CCA-IMT and CCA-AD increased considerably from the premenopausal stage to the perimenopausal and postmenopausal stage.[11] In a randomized trial, Wildman et al. implemented a 20-week lifestyle education program to women undergoing the menopausal transition. The program consisted of 15 group meetings led by nutritional and behavioral interventionalists and was aimed at reducing total and saturated fat intake, preventing weight gain, and increasing physical activity. Women in the intervention group had significantly lower body weights and significantly higher daily kilocalories of exercise after receiving the program compared with the control group. The intervention group also had a slower average progression of carotid IMT compared to the control group.[26] Thus, the midlife represents a critical window for CVD prevention, and women at this life stage may be particularly responsive to the beneficial effect of lifestyle prevention efforts.

We specifically focused on the three health behaviors that are largely modifiable while treating other physiological risk factors affected by lifestyle (i.e., BMI, blood pressure, blood lipids, and blood glucose) as covariates. Our study is thus different from previous work using the American Heart Association's Life's Simple 7 goals, which also include the management of BMI, blood pressure, blood glucose, and total cholesterol.[9] Even after adjusting for the lifestyle-related physiological risk factors, the adherence to a healthy lifestyle composed of abstinence from smoking, healthy diet, and regular engagement in physical activity is inversely associated with atherosclerosis in midlife women. Adherence to a healthy lifestyle was low in this study, as only 1.7% of the study population stayed in the top category for all three components at all three time points. This observation is consistent with the NHS, which reported that only 3% of the cohort

had a low-risk lifestyle.[80] The low prevalence of a healthy lifestyle in midlife women highlights the potential for lifestyle interventions aimed at this vulnerable population.

Abstinence from smoking showed the strongest inverse associations with all three measures of subclinical carotid atherosclerosis. Cigarette smoking is a major behavioral risk factor of atherosclerosis, through mechanisms including thrombosis, dyslipidemia, insulin resistance, vascular inflammation, abnormal vascular growth and angiogenesis, and loss of endothelial homeostatic and regenerative functions.[78, 131] Multiple studies have shown that prolonged smoking confers a higher CVD risk in women than in men.[132-135] Existing evidence is unclear regarding the associations of diet and physical activity with subclinical atherosclerosis. We found that diet quality may be associated with smaller CCA-AD and CCA-IMT, and that engagement in regular physical activity may be associated with smaller CCA-IMT. The observed associations for diet and physical activity lost statistical significance after the adjustment for other physiological risk factors, especially BMI. We thus speculate that BMI may partially mediate the potential beneficial effects of healthy diet and regular physical activity. We did not include BMI (or overweight/obesity status) as a separate component of the HLS but instead treated it as a covariate, because it is not a lifestyle behavior per se, but an intermediate health outcome affected by various other lifestyle factors. Formal causal mediation analysis is needed to quantitatively distinguish the direct effects of diet and physical activity and their indirect effects mediated by other physiological risk factors such as BMI. Because of the numerous exposures, outcomes, and models examined, this study was not completely free from multiple comparisons. We did not quantitatively correct the *P* values for multiple testing because all the models were based strictly on *a priori* hypotheses and the findings were interpreted with caution.

The current study has some important strengths. The use of repeated measures of behavioral risk factors allowed for a more accurate measurement of long-term lifestyle. Prior studies have shown that using cumulative exposures from repeated measurements may yield stronger associations with disease outcomes than using only the baseline exposure or the most recent exposure.[68, 69] In this study, the average HLS showed stronger associations with the outcomes compared to most of the visit-specific scores, though the findings were largely similar due to the relative stability of the HLS. The racial/ethnic composition of the study population was more diverse than most prior studies, as SWAN included not only non-Hispanic white women but also African American, Chinese, and Hispanic women, three groups that are underrepresented in the literature. We did not have sufficient statistical power to examine effect modification by race/ethnicity, so future multiethnic studies with larger sample sizes are needed to assess whether the association between lifestyle factors and subclinical atherosclerosis differs by racial and ethnic backgrounds.

This study also has some potential limitations. First and foremost, markers of subclinical carotid atherosclerosis were measured only once, at Visit 12/13. Without the baseline measures, we were unable to evaluate the change of subclinical atherosclerosis from baseline to Visit 12/13. Although we restricted the analyses to apparently healthy participants by excluding women with CVD at baseline or developed CVD during the follow-up, it is still possible that some women may already have substantial degrees of carotid atherosclerosis before baseline. Therefore, we could not pinpoint the midlife to be the most causally relevant period for the effect of lifestyle. Second, although the prospective nature of the exposures and the asymptomatic nature of the outcomes reduced the extent of reverse causation, it could not be completely ruled out. It was possible that some participants with subclinical atherosclerosis also subsequently had

other more detectable health conditions such as abnormal blood lipids or elevated blood pressure and would thus intentionally improve their lifestyle; this may partially explain the U-shaped association observed for the highest HLS level for which the outcome measures were similar or even higher than those observed for the second highest HLS level. Another possible reason for the U-shaped association is that some physiological covariates adjusted for in the full model, such as body mass index, blood pressure, blood glucose, and blood lipids, may partially mediate the effect for the highest HLS level, as the U-shaped tendency was not evident in the crude or partially adjusted models. Third, the exposure data inevitably had some measurement error, especially for the self-reported data of physical activity and dietary intake. However, both the physical activity questionnaire[121] and the FFQ[88, 89] used in SWAN have been validated in similar populations. Also, the use of up to three repeated measures of exposures considerably reduced the measurement error. Fourth, as the lifestyle data were not available after Visit 9, we were not able to capture the lifestyle exposures between Visit 9 and Visit 12/13. However, 74% of the participants were postmenopausal by Visit 9 so the average HLS from baseline to Visit 9 still reasonably represents the lifestyle during the menopausal transition.

In conclusion, the menopausal transition represents a crucial, yet understudied, window of increased cardiovascular risk in women. This prospective study provides evidence that a healthy lifestyle during the midlife, characterized by abstinence from smoking, a healthy diet, and engagement in regular physical activity, is associated with less subclinical atherosclerosis. We further documented that the prevalence of these healthy behaviors is extremely low in midlife women. This work highlights the growing recognition of the midlife as a critical window for CVD prevention and strongly supports the need for lifestyle interventions aimed at promoting these modifiable health behaviors in midlife women.

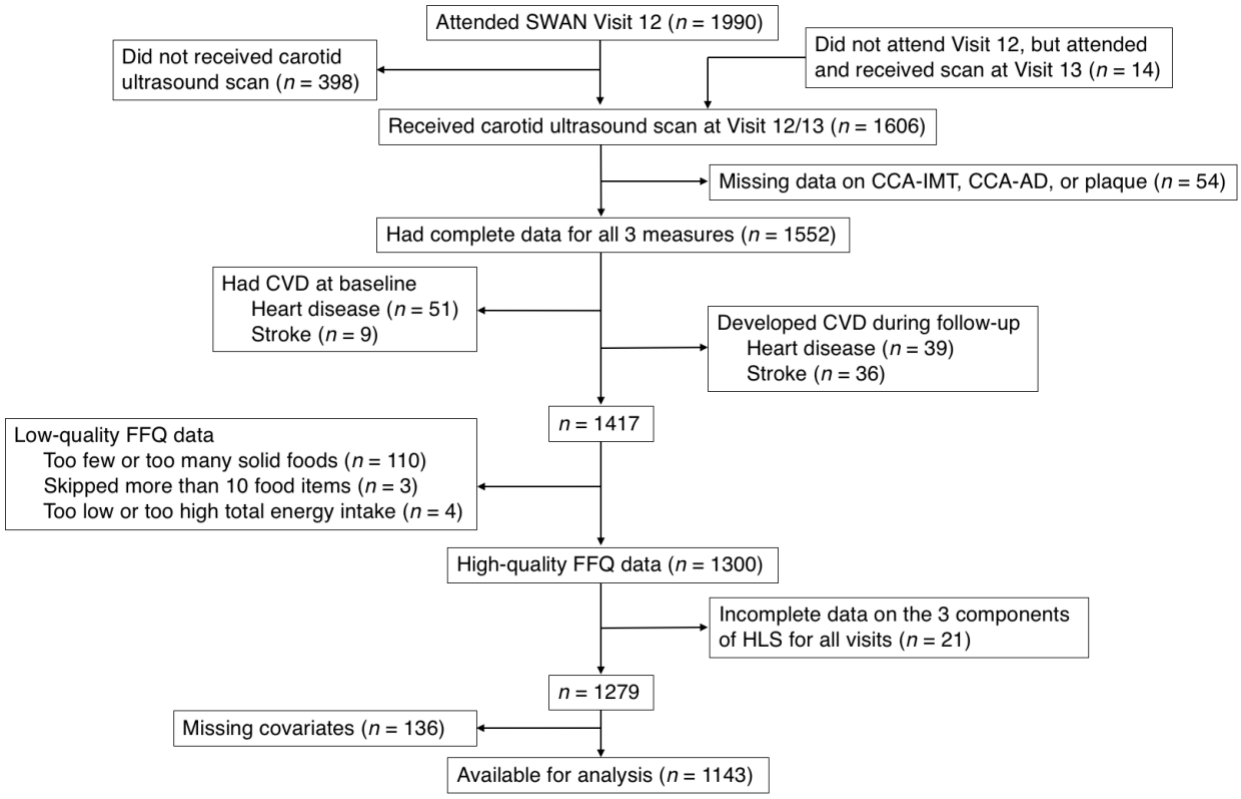

Figure 4.1 Exclusion flow of participants for the association between Healthy Lifestyle Score and subclinical carotid atherosclerosis in the Study of Women's Health Across the Nation. Abbreviations: CVD, cardiovascular disease; FFQ, food frequency questionnaire; HLS, Healthy Lifestyle Score.

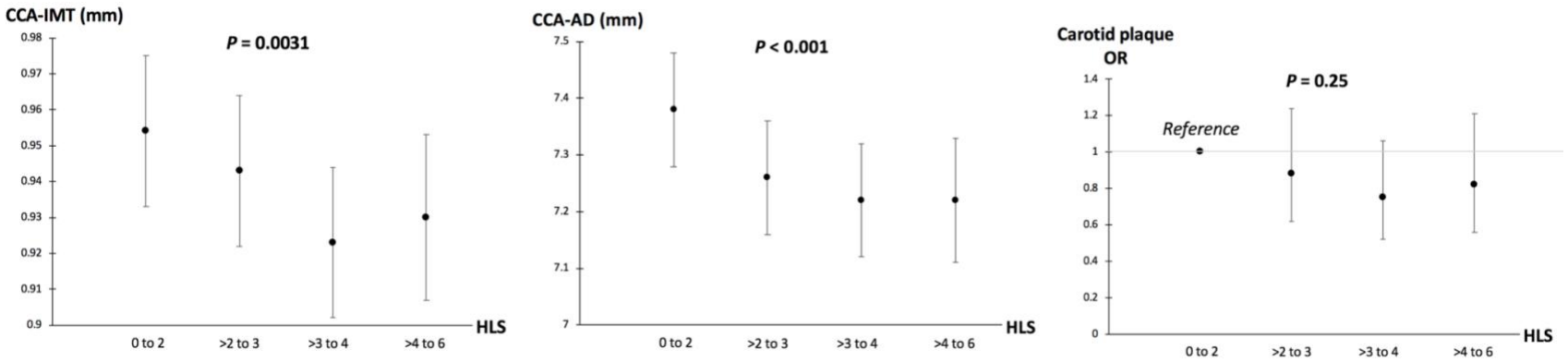

Figure 4.2 Average Healthy Lifestyle Score and subclinical carotid atherosclerosis among 1,143 participants of the Study of Women's Health Across the Nation**.** Values are least squares means (95% CIs) for CCA-IMT/CCA-AD from linear models and odds ratios (95% CIs) for carotid plaque (high vs. moderate vs. none) from cumulative logit models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). *P* values were computed by using the HLS as a continuous variable. Models were adjusted for age at the carotid scan (continuous), race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), total energy intake (continuous), menopausal status (premenopausal or early perimenopausal), use of hormone therapy during the follow-up (ever or never), hot flash at Visit 12 (binary), number of missing visits for HLS (0, 1, or 2), body mass index (continuous), elevated blood pressure (binary), elevated fasting glucose (binary), serum triglycerides (continuous), total cholesterol (continuous), high-density lipoprotein cholesterol (continuous), low-density lipoprotein cholesterol (continuous), use of antilipidemic medications (binary; Visit 12), and use of antihypertensive medications (binary; Visit 12). The baseline covariates were used unless otherwise specified. Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CI; confidence interval; HLS, Healthy Lifestyle Score; IMT, intima-media thickness; OR, odds ratio.

Table 4.1 Characteristics of the study population by category of average Healthy Lifestyle Score among 1,143 participants of the Study of Women's Health Across the Nation[a]

	Total	Categories of average HLS				
	n = 1143	0 to 2 (*n* = 256)	>2 to 3 (*n* = 293)	>3 to 4 (*n* = 306)	>4 to 6 (*n* = 288)	*P*-trend[b]
Major covariates						
Age at baseline[c], year	46.31 (2.66)	46.19 (2.59)	46.16 (2.60)	46.21 (2.66)	46.68 (2.75)	0.028
Age at the carotid scan[c], year	60.15 (2.70)	60.02 (2.65)	59.98 (2.65)	60.06 (2.70)	60.51 (2.77)	0.028
Race and ethnicity						< 0.001
- African American, %	26.16	34.19	24.25	22.01	18.59	
- Hispanic, %	5.77	8.10	8.55	5.23	0.61	
- Chinese, %	13.56	1.08	12.70	16.90	24.91	
- Non-Hispanic white, %	54.51	56.63	54.50	55.86	55.89	
Education level						< 0.001
- High school or less, %	20.65	32.95	21.55	17.24	11.97	
- Some college, %	30.45	38.34	35.52	29.91	19.71	
- College degree/post-college, %	48.91	28.70	42.93	52.85	68.32	
Somewhat/very hard to pay for basics, %	30.97	41.46	38.52	29.66	16.52	< 0.001
Marital status						< 0.001
- Single/never married, %	12.95	15.14	10.38	12.33	13.32	
- Married/living as if married, %	70.34	62.15	74.93	73.23	73.61	
- Separated/widowed/divorced, %	16.71	22.71	14.69	14.44	13.06	
Self-rated overall health						< 0.001
- Excellent/very good, %	64.48	52.86	60.20	65.45	77.99	
- Good, %	26.60	37.76	30.52	25.12	16.67	
- Fair/poor, %	8.92	9.38	9.28	9.43	5.34	
CES-D scale ≥16, %	21.78	32.29	24.57	22.08	12.47	< 0.001
Total energy intake, kcal/day	1817.91 (653.99)	1892.69 (674.23)	1777.98 (662.60)	1803.75 (592.21)	1826.43 (618.72)	0.79
Body mass index, kg/m²	27.91 (6.83)	29.52 (7.16)	28.83 (6.77)	27.65 (6.41)	25.09 (4.73)	< 0.001
Smoking status						
- Never, %	62.84	21.26	66.55	73.86	84.03	< 0.001
- Past, %	25.42	37.40	27.30	22.55	15.97	
- Current, %	11.74	41.34	6.14	3.59	0.00	
Menopausal status						0.0074
- Early Perimenopausal, %	43.66	50.24	44.78	38.46	35.97	
- Premenopausal, %	56.34	49.76	55.22	61.54	64.03	
Hormone therapy use (ever)[d], %	42.78	39.59	41.45	40.07	43.44	0.047
Self-reported hot flash[e], %	81.36	83.59	79.52	80.07	82.64	0.85
Number of missing visits						< 0.001
- 0, %	58.97	44.99	58.86	62.84	75.58	
- 1, %	26.16	33.39	27.51	24.49	16.73	
- 2, %	14.87	21.62	13.63	12.68	7.68	
Elevated blood pressure, %	26.16	28.89	23.13	28.02	19.55	0.0084

- Use of antihypertensive medications, %	10.85	11.33	11.95	10.46	9.72	0.47
Elevated fasting glucose, %	20.56	24.12	23.06	18.99	11.54	< 0.001
Serum triglycerides, mg/dL	104.85 (56.54)	116.38 (60.01)	109.20 (59.64)	98.19 (53.67)	97.25 (50.97)	< 0.001
- Elevated serum triglycerides, %	16.89	23.45	17.20	16.63	10.61	< 0.001
Total cholesterol, mg/dL	192.72 (33.24)	195.64 (34.56)	197.60 (33.76)	190.57 (32.09)	187.45 (31.90)	< 0.001
- Elevated total cholesterol, %	38.85	41.80	44.37	36.60	32.99	0.013
HDL cholesterol, mg/dL	56.57 (13.67)	52.13 (13.02)	56.20 (13.91)	57.98 (13.10)	59.39 (13.63)	< 0.001
- Reduced HDL cholesterol, %	32.63	46.09	33.45	28.43	24.31	< 0.001
LDL cholesterol, mg/dL	115.17 (29.84)	120.23 (31.26)	119.54 (30.44)	112.95 (28.22)	108.60 (28.18)	< 0.001
- Elevated LDL cholesterol, %	29.05	34.77	35.49	26.14	20.49	< 0.001
Non-HDL cholesterol (mg/dL)	136.15	143.51	141.39	132.59	128.06	< 0.001
Use of antilipidemic medications, %	0.26	0.78	0.34	0.00	0.00	0.11
Subclinical atherosclerosis						
CCA-IMT[e], mm	0.92 (0.14)	0.96 (0.15)	0.92 (0.12)	0.90 (0.13)	0.88 (0.12)	< 0.001
CCA-AD[e], mm	7.19 (0.66)	7.34 (0.66)	7.16 (0.62)	7.10 (0.60)	7.02 (0.58)	< 0.001
Carotid plaque[e, f]						0.038
- None, %	57.39	49.50	55.94	60.10	58.62	
- Moderate, %	18.29	17.47	15.34	18.08	22.67	
- High, %	24.32	33.04	28.72	21.82	18.71	

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CES-D scale, Center for Epidemiologic Studies Depression scale; HDL, high-density lipoprotein; HLS, Healthy Lifestyle Score; IMT, intima-media thickness; LDL, low-density lipoprotein.

[a] Values are means (standard deviations) for continuous variables and percentages for categorical variables. Values stratified by categories of average HLS are standardized to the baseline age distribution of the study population. Values of polytomous variables may not sum to 100% due to rounding. The variables are the baseline measures unless specified otherwise.

[b] Computed by linear models for continuous covariates and logistic models for binary/categorical covariates. The median HLS of a level was assigned to participants in the corresponding level and treated as a continuous variable.

[c] Values are not age-standardized.

[d] Ever use was defined as reported use at any visit from baseline to the visit of the carotid scan.

[e] Measured either at Visit 12 (2009-2011) or Visit 13 (2011-2013).

[f] None: carotid plaque index = 0; moderate: carotid plaque index = 1; high: carotid plaque index ≥ 2.

Table 4.2 Average Healthy Lifestyle Score and subclinical carotid atherosclerosis among 1,143 participants of the Study of Women's Health Across the Nation[a]

	0 to 2		>2 to 3		>3 to 4		>4 to 6		% of SD difference [b]	*P*
n (%)	256 (22.40)		293 (25.63)		306 (26.77)		288 (25.20)			
CCA-IMT, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	0.961	0.945, 0.978	0.933[e]	0.918, 0.949	0.904[g]	0.889, 0.920	0.898[g]	0.883, 0.914	-46%	< 0.001
Adjusted 1[c]	0.958	0.938, 0.979	0.942	0.923, 0.962	0.918[g]	0.898, 0.938	0.917[f]	0.895, 0.940	-30%	< 0.001
Adjusted 2[d]	0.954	0.933, 0.975	0.943	0.922, 0.964	0.923[f]	0.902, 0.944	0.930	0.907, 0.953	-17%	0.0031
CCA-AD, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	7.38	7.30, 7.46	7.19[g]	7.12, 7.27	7.12[g]	7.05, 7.19	7.08[g]	7.00, 7.15	-45%	< 0.001
Adjusted 1[c]	7.42	7.32, 7.52	7.28[f]	7.18, 7.37	7.22[g]	7.12, 7.32	7.19[g]	7.08, 7.29	-36%	< 0.001
Adjusted 2[d]	7.38	7.28, 7.48	7.26[e]	7.16, 7.36	7.22[f]	7.12, 7.32	7.22[f]	7.11, 7.33	-24%	< 0.001
Carotid plaque	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI		
Crude	1.00	*Ref*	0.84	0.61, 1.16	0.71[e]	0.51, 0.97	0.72	0.52, 1.00	-	0.024
Adjusted 1[c]	1.00	*Ref*	0.86	0.61, 1.20	0.67[e]	0.48, 0.95	0.68[e]	0.47, 0.98	-	0.024
Adjusted 2[d]	1.00	*Ref*	0.88	0.62, 1.24	0.75	0.52, 1.06	0.82	0.56, 1.21	-	0.25

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CI, confidence interval; HLS, Healthy Lifestyle Score; IMT, intima-media thickness; LS-mean, least squares mean; OR, odds ratio; SD, standard deviation.

[a] Values are least squares means (95% CIs) for CCA-IMT/CCA-AD from linear models and odds ratios (95% CIs) for carotid plaque index (high vs. moderate vs. none) from cumulative logit models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). *P* values were computed by using the HLS as a continuous variable.

[b] The difference in the least squares means (by percent of the standard deviation of CCA-IMT/CCA-AD in the study population) comparing the >4 to 6 group to the 0 to 2 group.

[c] Adjusted for baseline covariates, age at the carotid scan (continuous), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), hot flash at Visit 12 (binary), and the number of missing visits for HLS (0, 1, or 2). The baseline covariates include race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), total energy intake (continuous), menopausal status (premenopausal or early perimenopausal).

[d] Adjusted 1 plus body mass index (continuous), elevated blood pressure (binary), elevated fasting glucose (binary), serum triglycerides (continuous), total cholesterol (continuous), high-density lipoprotein cholesterol (continuous), low-density lipoprotein cholesterol (continuous), use of antilipidemic medications (binary), and use of antihypertensive medications (binary). The baseline values of these covariates were used except for antilipidemic and antihypertensive medications, for which the values at Visit 12 were used.

[e] $P<0.05$ (compared to the 0 to 2 group).

[f] $P<0.01$ (compared to the 0 to 2 group).

[g] $P<0.001$ (compared to the 0 to 2 group).

Table 4.3 Long-term smoking status and subclinical carotid atherosclerosis among 1,143 participants of the Study of Women's Health Across the Nation[a]

	Current smoking at some point		Past smoking		Never smoking		% of SD difference[b]	*P*-trend
n (%)	158 (13.82)		274 (23.97)		711 (62.20)			
CCA-IMT, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	0.980	0.959, 1.001	0.906[e]	0.890, 0.923	0.917[e]	0.907, 0.927	-46%	< 0.001
Adjusted[c]	0.976	0.952, 1.001	0.922[e]	0.899, 0.945	0.929[e]	0.912, 0.947	-34%	0.0026
CCA-AD, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	7.40	7.30, 7.50	7.15[e]	7.08, 7.23	7.15[e]	7.10, 7.20	-38%	< 0.001
Adjusted[c]	7.46	7.35, 7.58	7.26[d]	7.15, 7.37	7.22[e]	7.14, 7.31	-36%	< 0.001
Carotid plaque	OR	95% CI	OR	95% CI	OR	95% CI		
Crude	1.00	*Ref*	0.55[d]	0.38, 0.80	0.55[e]	0.40, 0.76	-	0.0028
Adjusted[c]	1.00	*Ref*	0.45[e]	0.30, 0.69	0.51[e]	0.35, 0.73	-	0.0064

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CI, confidence interval; IMT, intima-media thickness; LS-mean, least squares mean; OR, odds ratio; SD, standard deviation.

[a] Long-term smoking status was summarized using the available visits from baseline, Visit 5, and Visit 9. Values are least squares means (95% CIs) for CCA-IMT/CCA-AD from linear models and odds ratios (95% CIs) for carotid plaque index (high vs. moderate vs. none) from cumulative logit models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). *P*-trend values were computed by assigning 0, 1, and 2 to the three groups, respectively, while treating it as a continuous variable.

[b] The difference in the least squares means (by percent of the standard deviation of CCA-IMT/CCA-AD in the study population) comparing the "never smoking" group to the "current smoking at some point" group.

[c] Adjusted for baseline covariates, age at the carotid scan (continuous), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), hot flash at Visit 12 (binary), the number of missing visits for HLS (0, 1, or 2), long-term physical activity (consistently not meeting the recommendation, mixture of not meeting and partially meeting the recommendation, or fully meeting the recommendation at some point), and average alternate healthy eating index score (tertiles). The baseline covariates include race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), total energy intake (continuous), menopausal status (premenopausal or early perimenopausal), body mass index (continuous), elevated blood pressure (binary), elevated fasting glucose (binary), serum triglycerides (continuous), total cholesterol (continuous), high-density lipoprotein cholesterol (continuous), low-density lipoprotein cholesterol (continuous), use of antilipidemic medications (binary), and use of antihypertensive medications (binary). The baseline values of these covariates were used except for antilipidemic and antihypertensive medications, for which the values at Visit 12 were used.

[d] $P<0.01$ (compared to the "current smoking at some point" group).

[e] $P<0.001$ (compared to the "current smoking at some point" group).

Table 4.4 Average Alternate Healthy Eating Index and subclinical carotid atherosclerosis among 1,143 participants of the Study of Women's Health Across the Nation[a]

	Tertile 1		Tertile 2		Tertile 3		% of SD difference[b]	*P*-trend
n (%)	381 (33.33)		381 (33.33)		381 (33.33)			
CCA-IMT, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	0.948	0.934, 0.962	0.919[g]	0.905, 0.932	0.903[h]	0.889, 0.916	-33%	< 0.001
Adjusted 1[c]	0.952	0.932, 0.972	0.934	0.914, 0.953	0.928[f]	0.908, 0.948	-18%	0.019
Adjusted 2[d]	0.956	0.935, 0.977	0.941	0.921, 0.962	0.937	0.916, 0.958	-14%	0.067
Adjusted 3[e]	0.952	0.931, 0.973	0.938	0.918, 0.959	0.938	0.917, 0.959	-10%	0.18
CCA-AD, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	7.30	7.24, 7.37	7.15[g]	7.08, 7.21	7.10[h]	7.04, 7.17	-30%	< 0.001
Adjusted 1[c]	7.40	7.30, 7.49	7.29[f]	7.20, 7.39	7.26[g]	7.16, 7.36	-21%	0.0072
Adjusted 2[d]	7.40	7.30, 7.51	7.31[f]	7.21, 7.41	7.28[f]	7.18, 7.39	-18%	0.016
Adjusted 3[e]	7.37	7.27, 7.47	7.29	7.19, 7.38	7.29	7.19, 7.39	-12%	0.11
Carotid plaque	OR	95% CI	OR	95% CI	OR	95% CI		
Crude	1.00	*Ref*	0.87	0.66, 1.14	0.89	0.67, 1.17	-	0.40
Adjusted 1[c]	1.00	*Ref*	0.92	0.69, 1.23	0.91	0.67, 1.23	-	0.53
Adjusted 2[d]	1.00	*Ref*	1.00	0.74, 1.35	1.00	0.73, 1.37	-	0.99
Adjusted 3[e]	1.00	*Ref*	1.01	0.75, 1.35	1.02	0.74, 1.40	-	0.91

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CI, confidence interval; IMT, intima-media thickness; LS-mean, least squares mean; OR, odds ratio; SD, standard deviation.

[a] Average Alternate Healthy Eating Index score was calculated using the available visits from baseline, Visit 5, and Visit 9. Values are least squares means (95% CIs) for CCA-IMT/CCA-AD from linear models and odds ratios (95% CIs) for carotid plaque index (high vs. moderate vs. none) from cumulative logit models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). *P*-trend values were computed by assigning the median score of a level to participants in the corresponding level and treating it as a continuous variable.

[b] The difference in the least squares means (by percent of the standard deviation of CCA-IMT/CCA-AD in the study population) comparing the third tertile to the first tertile.

[c] Adjusted for baseline covariates, age at the carotid scan (continuous), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), hot flash at Visit 12 (binary), the number of missing visits for Healthy Lifestyle Score (0, 1, or 2), long-term smoking status (current smoking at some point, past smoking, or never smoking), and levels of long-term physical activity (consistently not meeting the recommendation, mixture of not meeting and partially meeting the recommendation, or fully meeting the recommendation at some point). The baseline covariates include race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), total energy intake (continuous), and menopausal status (premenopausal or early perimenopausal).

[d] Adjusted 1 plus elevated blood pressure (binary), elevated fasting glucose (binary), serum triglycerides (continuous), total cholesterol (continuous), high-density lipoprotein cholesterol (continuous), low-density lipoprotein cholesterol (continuous), use of antilipidemic medications (binary), and use of antihypertensive medications (binary). The baseline values of these covariates were used except for antilipidemic and antihypertensive medications, for which the values at Visit 12 were used.

[e] Adjusted 2 plus baseline body mass index (continuous).

[f] $P<0.05$ (compared to the first tertile).

[g] $P<0.01$ (compared to the first tertile).

[h] $P<0.001$ (compared to the first tertile).

Table 4.5 Long-term physical activity status and subclinical carotid atherosclerosis among 1,143 participants of the Study of Women's Health Across the Nation[a]

	Consistently not meeting the recommendation		Mixture of not meeting and partially meeting the recommendation		Fully meeting the recommendation at some point		% of SD difference[b]	*P*-trend
n (%)	268 (23.45)		517 (45.23)		358 (31.32)			
CCA-IMT, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	0.951	0.934, 0.967	0.926[f]	0.914, 0.938	0.898[g]	0.884, 0.912	-38%	< 0.001
Adjusted 1[c]	0.950	0.931, 0.970	0.937	0.918, 0.955	0.927	0.905, 0.950	-17%	0.059
Adjusted 2[d]	0.953	0.933, 0.974	0.941	0.922, 0.961	0.939	0.916, 0.963	-10%	0.26
Adjusted 3[e]	0.949	0.928, 0.970	0.940	0.920, 0.959	0.939	0.916, 0.962	-7%	0.43
CCA-AD, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	7.27	7.19, 7.35	7.22	7.16, 7.27	7.08[g]	7.01, 7.15	-28%	< 0.001
Adjusted 1[c]	7.34	7.24, 7.44	7.33	7.23, 7.42	7.28	7.17, 7.39	-9%	0.31
Adjusted 2[d]	7.35	7.25, 7.45	7.33	7.24, 7.43	7.32	7.21, 7.43	-4%	0.64
Adjusted 3[e]	7.31	7.21, 7.41	7.32	7.22, 7.41	7.32	7.21, 7.43	+1%	0.91
Carotid plaque	OR	95% CI	OR	95% CI	OR	95% CI		
Crude	1.00	*Ref*	0.77	0.58, 1.02	0.88	0.65, 1.19	-	0.53
Adjusted 1[c]	1.00	*Ref*	0.73[f]	0.53, 0.99	0.83	0.58, 1.19	-	0.47
Adjusted 2[d]	1.00	*Ref*	0.72[f]	0.52, 0.99	0.94	0.65, 1.36	-	0.98
Adjusted 3[e]	1.00	*Ref*	0.72[f]	0.53, 1.00	0.96	0.66, 1.38	-	0.92

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CI, confidence interval; IMT, intima-media thickness; LS-mean, least squares mean; OR, odds ratio; SD, standard deviation.

[a] Long-term physical activity status was summarized using the available visits from baseline, Visit 5, and Visit 9. Values are least squares means (95% CIs) for CCA-IMT/CCA-AD from linear models and odds ratios (95% CIs) for carotid plaque index (high vs. moderate vs. none) from cumulative logit models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). *P*-trend values were computed by assigning 0, 1, and 2 to the three groups, respectively, while treating it as a continuous variable.

[b] The difference in the least squares means (by percent of the standard deviation of CCA-IMT/CCA-AD in the study population) comparing the "fully meeting the recommendation at some point" group to the "consistently not meeting the recommendation" group.

[c] Adjusted for baseline covariates, age at the carotid scan (continuous), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), hot flash at Visit 12 (binary), the number of missing visits for HLS (0, 1, or 2), long-term smoking status (current smoking at some point, past smoking, or never smoking), and average alternate healthy eating index (tertiles). The baseline covariates include race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), total energy intake (continuous), and menopausal status (premenopausal or early perimenopausal).

[d] Adjusted 1 plus elevated blood pressure (binary), elevated fasting glucose (binary), serum triglycerides (continuous), total cholesterol (continuous), high-density lipoprotein cholesterol (continuous), low-density lipoprotein cholesterol (continuous), use of antilipidemic medications (binary), and use of antihypertensive medications (binary). The baseline values of these covariates were used except for antilipidemic and antihypertensive medications, for which the values at Visit 12 were used.

[e] Adjusted 2 plus baseline body mass index (continuous).

[f] $P<0.05$ (compared to the "consistently not meeting the recommendation" group).

[g] $P<0.001$ (compared to the "consistently not meeting the recommendation" group).

Supplemental Table 4.1 Alternate Healthy Eating Index scores among 1,143 participants in the Study of Women's Health Across the Nation

	Criteria for a minimum score of 0[a]	Criteria for a maximum score of 10[a]	AHEI scores[b] at baseline (1996-1997)	AHEI scores[b] at Visit 5 (2001-2003)	AHEI scores[b] at Visit 9 (2005-2007)
Vegetables (servings/day)	0	5	3.7 (2.4)	4.1 (2.5)	4.3 (2.5)
Fruit (servings/day)	0	4	3.4 (2.1)	3.2 (2.0)	3.1 (1.9)
Nuts and legumes (servings/day)	0	1	5.9 (2.7)	6.2 (2.7)	6.4 (2.8)
The ratio of white to red meat	0	4	4.3 (2.9)	4.5 (2.9)	4.7 (2.9)
Cereal fiber (g/day)	0	15	2.9 (1.4)	2.6 (1.3)	2.5 (1.4)
trans fat (% of energy)	≥4	≤0.5	3.7 (3.0)	4.1 (3.0)	4.4 (2.9)
P:S	≤0.1	≥1	6.4 (2.2)	6.6 (2.2)	6.7 (2.3)
Duration of multivitamin use[c]	<5y	≥5y	3.4 (1.9)	3.7 (2.2)	4.4 (2.4)
Alcohol (servings/day)	0 or >2.5	0.5-1.5	2.7 (3.7)	2.9 (3.8)	3.1 (3.9)
Total score (range)	2.5	87.5	36.4 (10.1) (11.0-69.6)	37.9 (9.9) (11.2-73.6)	39.5 (10.5) (15.1-74.3)

Abbreviations: AHEI, Alternate Healthy Eating Index; P:S, the ratio of polyunsaturated to saturated fatty acids.
[a] Intermediate values were scored proportionally between the minimum score and the maximum score.
[b] Values are mean (standard deviation).
[c] Duration of multivitamin intake was a binary component. The minimum score was 2.5, and the maximum score was 7.5.

Supplemental Table 4.2 Weighted Healthy Lifestyle Score and subclinical carotid atherosclerosis among participants of the Study of Women's Health Across the Nation[a]

	Quartile 1		Quartile 2		Quartile 3		Quartile 4		% of SD difference[b]	*P*
CCA-IMT, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	0.960	0.944, 0.976	0.930[f]	0.914, 0.946	0.904[g]	0.888, 0.919	0.898[g]	0.882, 0.914	-45%	< 0.001
Adjusted 1[c]	0.953	0.938, 0.977	0.936	0.916, 0.955	0.918[g]	0.897, 0.939	0.917[f]	0.894, 0.940	-29%	< 0.001
Adjusted 2[d]	0.953	0.933, 0.974	0.938	0.918, 0.959	0.923[f]	0.901, 0.945	0.930	0.906, 0.953	-17%	0.0035
CCA-AD, mm	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI	LS-mean	95% CI		
Crude	7.37	7.29, 7.45	7.20[f]	7.12, 7.27	7.11[g]	7.03, 7.18	7.07[g]	6.99, 7.14	-46%	< 0.001
Adjusted 1[c]	7.42	7.33, 7.52	7.29[e]	7.19, 7.39	7.19[g]	7.10, 7.29	7.19[g]	7.09, 7.30	-35%	< 0.001
Adjusted 2[d]	7.38	7.28, 7.48	7.28[e]	7.18, 7.38	7.19[g]	7.09, 7.29	7.23[f]	7.12, 7.34	-23%	< 0.001
Carotid plaque	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI		
Crude	1.00	*Ref*	0.75	0.55, 1.03	0.70[e]	0.51, 0.96	0.67[e]	0.49, 0.92	-	< 0.001
Adjusted 1[c]	1.00	*Ref*	0.75	0.54, 1.05	0.70[e]	0.50, 0.97	0.67[e]	0.48, 0.94	-	< 0.001
Adjusted 2[d]	1.00	*Ref*	0.75	0.54, 1.06	0.68[e]	0.48, 0.96	0.68[e]	0.48, 0.96	-	< 0.001

Abbreviations: AD, adventitial diameter; CCA, common carotid artery; CI, confidence interval; IMT, intima-media thickness; LS-mean, least squares mean; OR, odds ratio; SD, standard deviation.

[a] The weighted Healthy Lifestyle Score was generated by using the percentage of the coefficient of each individual component to the sum of the coefficients in the models with all components included. Physical activity was included both as a linear and a quadratic term in the creation of the weighed Healthy Lifestyle Score. Values are least squares means (95% CIs) for CCA-IMT/CCA-AD from linear models and odds ratios (95% CIs) for carotid plaque index (high vs. moderate vs. none) from cumulative logit models. The least square means were computed using sample mean values for continuous covariates and equal distributions for categorical covariates (i.e., the same percentage for each category of the covariate). *P* values were computed by using the score as a continuous variable.

[b] The difference in the least squares means (by percent of the standard deviation of CCA-IMT/CCA-AD in the study population) comparing highest quartile to the lowest quartile.

[c] Adjusted for baseline covariates, age at the carotid scan (continuous), use of hormone therapy from baseline to the visit of the carotid scan (ever or never), hot flash at Visit 12 (binary), and the number of missing visits for HLS (0, 1, or 2). The baseline covariates include race/ethnicity (African American, Hispanic, Chinese, or non-Hispanic white), education level (≤ high school, some college, or college degree/post-college), financial strain (somewhat/very hard paying for basics, or not hard paying for basics), marital status (single/never married, married/living as if married, or separated/widowed/divorced), self-rated overall health (excellent/very good, good, or fair/poor), Center for Epidemiologic Studies Depression scale (≥16 or <16), total energy intake (continuous), menopausal status (premenopausal or early perimenopausal).

[d] Adjusted 1 plus body mass index (continuous), elevated blood pressure (binary), elevated fasting glucose (binary), serum triglycerides (continuous), total cholesterol (continuous), high-density lipoprotein cholesterol (continuous), low-density lipoprotein cholesterol (continuous), use of antilipidemic medications (binary), and use of antihypertensive medications (binary). The baseline values of these covariates were used except for antilipidemic and antihypertensive medications, for which the values at Visit 12 were used.

[e] $P<0.05$ (compared to the first quartile).

[f] $P<0.01$ (compared to the first quartile).

[g] $P<0.001$ (compared to the first quartile).

Chapter 5. Conclusions

5.1 Summary of Findings

This dissertation examines the prospective associations of various lifestyle factors during the midlife, including beverage intake, dietary patterns, and overall lifestyle, with subclinical carotid atherosclerosis later in life among women enrolled in the Study of Women's Health Across the Nation (SWAN).

In Chapter 2 (Aim 1), we evaluated the prospective associations between the intakes of eight groups of beverages during the midlife and measures of subclinical carotid atherosclerosis among 931 women. We found an inverted J-shaped association between coffee intake and CCA-IMT; compared to non-drinkers, women with occasional coffee intake ($\leq$ 2 cups per day) had a higher CCA-IMT whereas women with moderate-to-heavy intake (> 4 cups per day) might have a lower CCA-IMT. We also found an inverse linear association between alcoholic beverage intake and CCA-IMT within the range of moderate intake (< 2 drinks per day).

In Chapter 3 (Aim 2), we used repeatedly collected dietary data to assess the prospective associations of empirical dietary patterns during the midlife with measures of subclinical carotid atherosclerosis later in life among 1,246 women. We compared three variable reduction techniques, including principal component analysis, reduced rank regression, and partial least squares regression, in the estimation of diet-atherosclerosis associations. We identified a Western dietary pattern from each method and reported that the adherences to the three Western patterns were all positively associated with CCA-IMT. This study suggests that the positive association

between the Western diet and CCA-IMT was robust under different dietary pattern derivation methods. The adoption of a diet low in red meat, processed meat, deep fried products, and sugar-sweetened beverages among midlife women may protect against future atherosclerosis.

In Chapter 4 (Aim 3), we created a composite midlife Healthy Lifestyle Score (HLS) from three behavioral cardiovascular risk factors that are mostly modifiable (smoking, diet quality, and physical activity) to estimate the prospective association between the HLS and measures of subclinical carotid atherosclerosis among 1,143 women. We also examined the independent association between each component of the HLS and subclinical carotid atherosclerosis. We documented that the prevalence of these healthy behaviors was extremely low in midlife women. We found that the level of HLS during the midlife was associated with smaller CCA-IMT and CCA-AD. Among the three individual components of the HLS, abstinence from smoking displayed the strongest inverse associations with all three measures of subclinical carotid atherosclerosis.

5.2 Strengths and Limitations

This dissertation has several strengths. First, we specifically focused on midlife women undergoing the menopausal transition, which is an understudied population group that may be especially amenable to lifestyle interventions. Second, the racial/ethnic composition of the study population was more diverse than many prior studies of women's health, as the participants of the SWAN included not only Caucasian women but also the underrepresented groups of African American, Chinese, and Hispanic women. Third, we used repeatedly collected measures of lifestyle factors to construct the midlife exposures, which reduced the random within-person variation and increased the accuracy of the measurement of long-term lifestyle.[58] Indeed, prior studies have shown that using cumulative exposures from repeated measures over time may yield stronger associations with cardiometabolic outcomes than using only the baseline exposure or the most recent exposure.[68, 69] Fourth, the use of multiple dietary pattern derivation techniques provided a rare opportunity to compare the performances of different dimension reduction methods in the estimation of diet-disease associations. Fifth, the robustness of the key findings was supported by extensive sensitivity analyses that addressed potential selection bias, information bias, and unmeasured and time-varying confounding.

This dissertation also has some limitations. First and foremost, measures of subclinical carotid atherosclerosis were collected only once, at Visit 12/13. Without any baseline measures, we could not evaluate the change in subclinical atherosclerosis from baseline to Visit 12/13. Although we restricted the analyses to apparently healthy participants by excluding women with CVD at baseline or developed CVD during the follow-up, it was still possible that some women might already have accumulated substantial degrees of carotid atherosclerosis before the midlife. Therefore, we could not pinpoint the midlife to be the most causally relevant period for the effect

of lifestyle factors. Second, the exposure data inevitably had some measurement error, especially for the self-reported dietary data. However, the FFQ[88, 89] used in the SWAN has been previously validated in similar populations. Also, the exclusion of participants with low-quality FFQ data and the use of average exposure measures collected over 10 years reduced the random measurement error and improved the ascertainment of long-term exposures. Third, although the prospective nature of the exposures and the asymptomatic nature of the outcomes reduced the extent of reverse causation, it could not be completely ruled out. It was possible that some participants with subclinical atherosclerosis also had other more detectable health conditions and would thus intentionally improve their lifestyle. Fourth, in the beverage intake analyses, we could not separately examine the effects of extremely high intakes for some beverages (e.g., > 3 cups per day of tea or > 2 servings per day of SSB) because such extreme values were rare in the study population. The relatively low variability of some beverages may partially account for the lack of observed associations for those beverages. Fifth, we had no information on coffee brewing methods or decaffeinated coffee intake. Sixth, in the dietary pattern analyses, CRP and PAI-1 may not represent the inflammatory and hemostatic processes that are most pertinent to the development of atherosclerosis, in which case the patterns derived by RRR and PLS may underestimate the true associations. Seventh, although we extensively adjusted for covariates, it was not possible to eliminate residual confounding due to the observational nature of the SWAN cohort. Residual confounding is especially a concern for the beverage intake analysis of Chapter 2, which is particularly susceptible to unmeasured confounding by other dietary, sociodemographic, and health factors. For example, the associations for certain beverages may still be confounded by other dietary factors. However, we used the AHEI, a well-established dietary index that incorporates a wide range of important foods and nutrients and the types of

protein and fats,[94, 95] to adjust for the overall diet. Last but not least, due to the large number of exposures and outcomes examined, multiple testing may be an issue. After controlling for multiple comparisons, the key associations in the dietary pattern analysis remained significant or marginally significant, whereas none of the associations observed in the beverage intake analysis stayed significant. However, the analyses were based on *a priori* hypotheses and reported either in the main text or the supplemental tables. Also, the key findings were robust under extensive sensitivity analyses. Nevertheless, some of the observed associations might be due to chance alone, and the results should be interpreted with caution. Future studies are needed to confirm our findings.

5.3 Public Health Implications

The menopausal transition in women is associated with higher risk of cardiovascular disease,[1-3, 136, 137] as well as a series of unfavorable changes in cardiometabolic risk factors independently of chronological aging, such as increased total abdominal fat and visceral fat,[4, 5] elevated total cholesterol, LDL cholesterol, and apolipoprotein B,[6] and reduced cardioprotective effect of HDL cholesterol.[7, 8] The menopause-induced cardiovascular risk may be counteracted by improvements in lifestyle factors during the menopausal transition.[138] Therefore, the midlife represents a crucial, yet understudied, window of cardiovascular assessment and intervention in women. However, few prior studies have specifically evaluated the potential effects of lifestyle factors during the midlife on cardiovascular risk later in life. To the best of our knowledge, this dissertation is the first to use repeated and prospective measures of lifestyle factors to assess the impacts of lifestyle during the menopausal transition on subclinical carotid atherosclerosis later in life. This dissertation contributes to a better understanding of the role of lifestyle factors on cardiovascular risk in the understudied population of midlife women and highlights the growing recognition that the midlife is a critical period for cardiovascular prevention in women.

The benefits and risks of coffee intake have been the subjects of contentious debate for decades. According to the recommendations from the USDA/HHS 2015-2020 *Dietary Guidelines for Americans*, moderate coffee consumption (three to five 8-oz cups of coffee per day, equivalent to 400 mg of caffeine per day) can be incorporated into healthy eating patterns.[139] This guidance is informed by strong and consistent evidence showing that, in healthy adults, moderate coffee consumption is not associated with an increased risk of major chronic diseases or premature death, especially from CVD.[139] Recent evidence for clinical endpoints (mortality and clinical morbidity) indicated that coffee intake equivalent to 400 mg/day of

caffeine may actually be too conservative, and that consuming even more than 4 cups of coffee per day would still be safe and potentially beneficial.[140] Our results are consistent with the current recommendation as women who consumed more than 4 cups of coffee per day had a lower CCA-IMT compared to women who did not drink coffee. The inverted J-shaped association indicates that occasional coffee intake of no more than 2 cups per day during the midlife is associated with more subclinical atherosclerosis. This finding suggests that the harmful effects of coffee constituents may outweigh the beneficial properties among light drinkers, which is in line with previous evidence on clinical endpoints showing that daily intake of 3 to 5 cups of coffee confers the maximal cardiovascular benefits. This dissertation contributes to the emerging literature that moderate and even heavy coffee intake can be part of a healthy lifestyle.

The health implication of alcohol drinking is also among the most controversial issues in nutritional epidemiology. The USDA/HHS 2015-2020 *Dietary Guidelines for Americans* recommends an upper limit of 98 grams per week of alcohol for women and an upper limit of 196 grams per week for men, which corresponds to no more than one drink per day for women and no more than two drinks per day for men.[139] Similarly, the Alternate Healthy Eating Index recommends 0.5 to 1.5 drinks per day for women, and 0.5 to 2 drinks per day for men.[141] In line with these previous recommendations, we found that alcoholic beverages were inversely and linearly associated with CCA-IMT within the range of moderate intake (less than two drinks per day). The potential effect of heavy alcohol intake on subclinical atherosclerosis cannot be reliably evaluated in this dissertation as the study population had few women (less than 4%) with more than two servings per day of alcohol intake. It is likely that alcoholic beverages are protective against atherosclerosis when consumed in moderation but will have a detrimental effect when heavily consumed. Despite the relatively consistent epidemiologic evidence on the

cardiovascular benefit of moderate alcohol intake, future public-health guidelines to recommend moderate alcohol intake should be made cautiously due to the concern of binge drinking and alcohol abuse. Currently, it is not recommended that individuals initiate alcohol consumption for cardiovascular benefit, and for those already drinking, consumption should be limited to recommended amounts and preferably consumed with meals.[142] Furthermore, the risks of cancers and diseases of other organ systems need to be weighed carefully when making recommendations. Therefore, individualized consultation on alcohol intake will perhaps be more suitable than population-level recommendations.

People do not consume foods and beverages in isolation but rather in the combination typically referred to as "eating patterns" or "dietary patterns." Therefore, it is also paramount to examine the totality of the whole diet to account for the complex and synergistic relationships among dietary components. While previous dietary guidelines focused primarily on individual foods and nutrients, the most recent USDA/HHS 2015-2020 *Dietary Guidelines for Americans* starts focusing on healthy eating patterns.[139] It is worth noting that a healthy dietary pattern is not a rigid prescription but an adaptable framework that can be tailored to individuals' personal and sociocultural preferences while fitting within their budget. The 2015-2020 Dietary Guidelines recommends reducing intake of sodium, added sugar, and saturated fats, while providing three examples of healthy dietary patterns that are compatible with this recommendation, including the Healthy U.S.-Style Eating Pattern, the Healthy Mediterranean-Style Eating Pattern, and the Healthy Vegetarian Eating Pattern.[139] This dissertation provides evidence that the Western dietary pattern characterized by high intakes of red meat, processed meat, deep fried products, and sugar-sweetened beverages, and low in fruits, vegetables, and legumes, is a risk factor for subclinical atherosclerosis in women. The Prudent dietary pattern *per se*, with relatively high

loadings on fruits, vegetables, and legumes, is not associated with subclinical atherosclerosis in this dissertation. However, this lack of association may be explained by the overall low level of healthy foods intake of the American diet. Indeed, the Healthy Eating Index scores of Americans have remained low for a long time.[139] Future dietary guidelines and lifestyle interventions for cardiovascular health should focus on increasing fruits, vegetables, and legumes intake while reducing the intake of foods high in sodium, added sugar, saturated fat, and refined grains.

The dissertation supports that physical activity and smoking are also crucial modifiable behavioral risk factors for atherosclerosis. About $117 billion in annual health care costs and about 10% of premature mortality are associated with inadequate physical activity.[79] The recently updated *Physical Activity Guidelines for Americans* recommend that adults should pursue 150 minutes to 300 minutes per week of moderate-intensity aerobic physical activity, or 75 minutes to 150 minutes per week of vigorous-intensity aerobic physical activity (or an equivalent combination of both) for substantial health benefits.[79] It is important to recognize that, regarding physical activity, anything is better than nothing; being less sedentary and doing any amount of moderate-to-vigorous physical activity can provide health benefits. Also, physical activity does *not* need to be in bouts of at least 10 minutes; bouts of any length contribute to the health benefits associated with the accumulated volume of physical activity. Furthermore, additional health benefits can be gained beyond the recommended levels.[79] Despite the relatively consistent literature on the health benefits of moderate-to-vigorous physical activity, the physical activity levels of the Americans remained far below the recommendation. It is estimated that only 19% of American women are meeting the physical activity guidelines.[79] This dissertation shows that only a very small proportion of midlife women could maintain a healthy lifestyle

throughout the menopausal transition. We also provide evidence for the protective effect of a healthy midlife lifestyle against future atherosclerosis.

To make a positive impact on women's cardiovascular health at the population level, we need more than epidemiologic results and lifestyle guidelines. Preventive interventions need to be applied in both the clinical setting and the community setting. The awareness of cardiovascular disease prevention in women remains low. It is estimated that less than half of the women know that CVD is the top killer of women; only 40% of routine care in women includes a heart risk check, and only 22% of primary care physicians feel well-prepared to assess cardiovascular risk in women.[143] Practicing clinicians should emphasize the importance of the midlife as a crucial stage for cardiovascular prevention and the benefits of improving the overall lifestyle, even among women who are low-risk and apparently healthy without any clinically diagnosed CVD yet. Population-level interventions should be implemented to not only facilitate lifestyle changes but also increase long-term adherence. We return to this issue toward the end of next section.

5.4 Future Research Directions

This dissertation contributes new information to the impacts of lifestyle factors, including beverage intake, dietary patterns, and overall lifestyle, on subclinical atherosclerosis in the understudied population of midlife women. Several directions could be taken in future research to build upon our findings.

First, a major limitation of this dissertation is the lack of baseline measures of atherosclerosis. Without the baseline measures, we were unable to evaluate the change of subclinical atherosclerosis over the course of the menopausal transition. Consequently, we could not reliably pinpoint the midlife to be the most causally relevant period for the effect of lifestyle factors. Carotid ultrasonography is a non-invasive technique of measuring atherosclerosis and is commonly used in research settings. Therefore, future prospective cohort studies could collect repeated measures of carotid atherosclerosis and lifestyle exposures over the menopausal transition to evaluate the longitudinal associations.

Second, while existing dietary guidelines often have relatively specific recommendations about solid foods intakes, such as fruits, vegetables, grains, dairy, and sources of protein,[139] guidelines on beverage intake are relatively lacking. While the harmful effects of sugar-sweetened beverages are well-established, the cardiovascular effects of coffee, tea, artificially sweetened beverages, fruit juices, milk especially low-fat or fat-free milk, and alcoholic beverages are still rather controversial. While evidence from randomized controlled trials is not always available, more prospective cohort studies with long-term follow-ups are needed to confirm our findings, to understand better the potential causal effects of beverage intake, and to make more comprehensive public-health recommendations on beverage intake. As the effects of some beverages (e.g., coffee and alcoholic beverages) may not be strictly linear, future work

should focus on the determination of the optimal intake levels of commonly consumed beverages for maximum cardiovascular benefits, both in the general population and among women undergoing the menopausal transition specifically.

Third, Chapter 3 (Aim 2) shows that, among the three Western dietary patterns derived by principal component analysis (PCA), reduced rank regression (RRR), and partial least squares regression (PLS), the PLS-derived pattern has the strongest association with the outcomes, perhaps through pro-inflammatory and pro-thrombotic pathways.[60, 61] PLS is a compromise between PCA and RRR, with the capability of identifying food combinations that are not only commonly consumed in the population but also associated with intermediate risk factors.[74] Our results indicate that PLS is a versatile technique that offers more flexibility over both PCA and RRR, especially in exploratory analyses in the presence of uncertainty about the mechanistic pathways.[75] PLS has been underutilized in the nutritional epidemiologic literature compared to PCA and RRR. Future studies with a comprehensive panel of potential intermediate response variables can take advantage of this technique to uncover the convoluted interrelationships among diet, intermediate risk factors, and disease outcomes. Combining results from such epidemiologic studies with evidence from *in vitro* and *in vivo* studies will deepen our understanding on how dietary patterns and their nutrient components influence cardiovascular risks, and shed light upon potential targets for prevention strategies.

Fourth, future work could focus on the examination of the interaction between lifestyle and race/ethnicity. This dissertation includes women from multiple racial and ethnic backgrounds, including non-Hispanic white, African American, Hispanic, and Chinese women, so the results are perhaps more generalizable to the U.S. population than those from many prior studies. Future multiethnic studies with adequate sample sizes within each racial/ethnic group

could also assess whether the effects of lifestyle factors on subclinical atherosclerosis may differ by racial and ethnic backgrounds.

Fifth, lifestyle behaviors, especially dietary practices, are heavily influenced by cultural backgrounds. For example, while the traditional Chinese diet consists of low to moderate intakes of meat and high intakes of vegetables, it also has high levels of refined grains (such as rice and noodles) and food processing. The dietary patterns specific to Chinese American women are unclear. Therefore, future research could aim to evaluate the composition and the cardiovascular effects of racial/ethnic-specific lifestyle patterns, such as racial/ethnic-specific dietary patterns.

Sixth, the potential effects of non-traditional lifestyle factors warrant further research, both among the general population and in midlife women specifically. Some examples of novel lifestyle-related exposures include sleep hygiene and the use of stress management techniques. Emerging evidence indicates that sleep duration[144-148] and the use of relaxation techniques such as meditation[149, 150] may be associated with cardiometabolic outcomes. More studies are needed to confirm their causal effects and to incorporate them into healthy lifestyle indices.

Last but certainly not least, epidemiologic evidence alone is futile if the knowledge learned is not implemented in the population. There is a large research gap in how lifestyle intervention programs should be applied to help individuals make and maintain lifestyle changes. Lifestyle education programs can often improve health behaviors in the short term, but the changes are typically difficult to maintain in the long run.[151] Future implementation research should address the issue of long-term adherence by enhancing motivation and understanding obstacles, and possibly by employing emerging techniques such as mobile health and wearable devices.[152] Changing the sociocultural environment to make the default lifestyle options healthier is also crucial. This endeavor will require conversations and collaborations among

epidemiologists, nutritionists, health educators, health informatics professionals, and policymakers. Constantly renewing evidence from nutritional and chronic disease epidemiologic research will play a pivotal role in improving people's dietary intake, physical activity, overall lifestyle, and ultimately, preventing diseases and prolonging life.

5.5 Conclusions

This dissertation evaluates the prospective associations of lifestyle factors during the midlife with subclinical carotid atherosclerosis later in life among women. We found that moderate intake of alcoholic beverages and moderate-to-heavy intake of coffee were associated with less atherosclerosis. A Western diet high in red meat, processed meat, deep fried products, and sugar-sweetened beverages was positively associated with atherosclerosis. Finally, adopting a heart-healthy lifestyle, especially abstaining from cigarette smoking, is vital for the prevention of atherosclerosis. This dissertation highlights the midlife as a crucial window for primary prevention of cardiovascular disease in women. Future research should address the obstacles to leading a healthy lifestyle in midlife women and the implementation of population-level interventions that can result in long-term improvements in modifiable health behaviors.

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