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Dietary Flavonoids in Cardiovascular and Cognitive Health

By

Margarethe Elisabeth Goetz

Doctor of Philosophy

Epidemiology

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Dietary Flavonoids in Cardiovascular and Cognitive Health

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An abstract of
A dissertation submitted to the Faculty of the
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2016

Abstract

Dietary Flavonoids in Cardiovascular and Cognitive Health

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Flavonoids are bioactive polyphenolic compounds found ubiquitously in vascular plants, which likely have pleiotropic effects, including anti-oxidant and anti-inflammatory activity. The role of dietary flavonoids in cardiovascular and cognitive health has been a topic of research interest for nearly two decades, with recently increasing interest. While progress has been made, existing literature is limited by incomplete dietary flavonoid information and lack of geographic and racial/ethnic diversity of studied populations. Geographic and racial disparities in cardiovascular and cognitive health emphasize the need to address these knowledge gaps, which is the primary objective of this dissertation.

We used data from the REasons for Geographic and Racial Differences in Stroke Study (REGARDS) for all three research aims. In the first study we examined the association between total flavonoid and flavonoid subclass intakes and incident ischemic stroke. Greater flavanone intake, primarily consumed from citrus fruits, was associated with a lower relative risk of incident ischemic stroke. We also found that non-Hispanic black participants in the REGARDS study were less likely to consume flavonoids in general, as compared to their white counterparts, except for flavanones, which black participants consumed more. Stroke belt residents were more likely to reported higher consumption of flavan-3-ols and lower consumption of anthocyanidins, flavones and flavanones than those living outside of the stroke belt. In the second study, we reported that greater anthocyanidin and greater proanthocyanidin intakes were each associated with a lower relative risk of CHD events. Finally, in the third study we found that anthocyanidins, flavones and flavonols were positively associated with global cognitive performance over time. These associations were not modified by sociodemographic factors, including race or region of residence.

The findings of this dissertation project extend the results of previous flavonoid research to non-Hispanic black Americans and Americans across the continental U.S. Specific suggestions about flavonoid subclass consumption are impractical given the complexity of the flavonoid content of foods. Our results highlight the importance of consuming a variety of plant-based foods on a daily basis. Interventional studies designed to test optimal combinations and quantities of flavonoids and flavonoid-rich foods are warranted.

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CHAPTER 1: INTRODUCTION

Cardiovascular disease (CVD) has been the leading cause of death in the United States since 1935.[1] By 2030, 40.5% of the U.S. population is projected to suffer from some form of CVD with ballooning direct and indirect economic costs.[2] Given this persistent burden of CVD, primary prevention of stroke, myocardial infarction (MI) and other forms of CVD remains critical. The prevalence of cognitive impairment and dementia is also expected to rise as the population ages, with an estimated 115 million cases of dementia worldwide by 2050.[3] With no curative treatment options and only moderately effective symptomatic therapies available for dementia, preventive strategies, such as diet, are needed. Like CVD, cognitive decline may be mediated by inflammation and oxidative stress. Traditional cardiovascular risk factors are associated with increased risk of cognitive decline.[4] Cognitive decline may begin in midlife years and CVD risk factors present in midlife are associated with cognitive decline in later years.[5,6]

Nutritional factors that prevent or slow the progression of CVD and cognitive decline may also help to improve and prolong quality of life as well as optimize cognitive function in older adults. The consumption of diets high in plant-based foods has been associated with decreased risk of incident CVD and cognitive decline.[7-12] Flavonoids are bioactive, polyphenolic compounds widely distributed in plants and may mediate the beneficial properties of plant-based diets for cardiovascular and cognitive health.[13] Proposed cardioprotective mechanisms for flavonoids include antioxidant and anti-inflammatory action, modulation of lipid metabolism and platelet function and attenuation of hypertension, as well as protection against neuroinflammation and promotion neuroplasticity.[14,15] Consistent with proposed biologic mechanisms, results

from epidemiologic studies suggest a protective effect for flavonoids against cardiovascular disease (CVD) mortality [13,16,17], incident coronary heart disease (CHD) [18,19], and incident stroke [20] as well as other indicators of cardiovascular risk, such as arterial stiffness, incident hypertension, and type 2 diabetes. [21-24] While dietary flavonoid research has progressed over the past decade, existing literature is limited by incomplete dietary flavonoid information and lack of geographic and racial/ethnic diversity of studied populations.

A lack of comprehensive dietary flavonoid composition tables has especially limited previous research. The use of diet composition tables with missing or incomplete information of flavonoid content in some foods or mixed dishes may have led to underestimation of flavonoid intake. Conversely, imputation of values in cooked dishes from raw foods, without adjusting for processing losses, may have led to overestimation. To address these limitations, the U.S. Department of Agriculture (USDA) released the Provisional Flavonoid Addendum. The Provisional Flavonoid Addendum provides flavonoid values for an expanded number (over 7,000) of foods and beverages, and uses more precise estimation methods for flavonoid content in mixed and cooked dishes [25].

A second limitation of existing literature is a general lack of racial and regional diversity in published studies. Black Americans and residents of the Southeastern United States, a region also known as the Stroke Belt, bear a higher burden of cardiovascular disease and cognitive impairment than whites and those living elsewhere in the U.S.[26-30] A variety of nontraditional risk factors, including dietary habits, may contribute to these disparities. In particular, fruit, vegetable and flavonoid intake differ by race.[31-35] Non-Hispanic blacks tend to consume fewer servings of fruits and vegetables than non-

Hispanic white counterparts and total flavonoid consumption is also lower among non-Hispanic black Americans as compared to their white Americans. Race and region of residence also have a synergistic effect on nutrient consumption, though less is known about regional consumption of flavonoids.[36] Therefore, differences in dietary flavonoid intake may help to explain racial and regional differences in CVD and cognitive decline. However, no studies of flavonoid intake and incident CVD or cognitive impairment have included a racially or geographically diverse cohort and examined differences by race and region

The goals for this dissertation project are to 1) evaluate the association between flavonoid intake and incident stroke and incident acute CHD in a biracial, national cohort, 2) evaluate the association between flavonoid intake and cognitive function over time, 3) to examine flavonoid intake by race and by geographic region, and 4) to assess potential effect modification of observed flavonoid intake-disease associations by race and region of residence.

Specific Aim #1

Examine the association of reported dietary total flavonoid and flavonoid subclass intakes with incident ischemic stroke in a biracial, national cohort. The approach to this aim was to use Cox proportional hazards models to explore this relationship using data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. Previous studies have been conducted in mainly white and geographically limited populations in the U.S. The hypothesis for this aim is that higher flavonoid intake is associated with lower relative risk of incident ischemic stroke and that the association does not differ by race or by region of residence.

Specific Aim #2

Examine the association of reported dietary total flavonoid and flavonoid subclass intakes with incident acute coronary heart disease (CHD) in a biracial, national cohort. The approach to this aim was to use Cox proportional hazards models to explore this relationship using data from the REGARDS study. Previous studies have been conducted in mainly white and geographically limited populations in the U.S. The hypothesis for this aim is that higher flavonoid intake is associated with lower relative risk of incident CHD and that the association does not differ by race or by region of residence.

Specific Aim #3

Examine the association between reported dietary total flavonoid and flavonoid subclass intakes with cognitive function in a biracial, national cohort. The approach to this study was to estimate the effect of flavonoids over time, on a composite cognitive score using mixed linear regression, allowing for correlation within participant cognitive measures over time and allowing for varying numbers of cognitive assessments. Data will come from the REGARDS study. The hypothesis for this aim is that greater flavonoid intake is associated with better cognitive performance scores and that the rate of change in decline in cognitive performance is slower those with greater flavonoid intake. Associations will not differ by race or region of residence.

CHAPTER 2: BACKGROUND

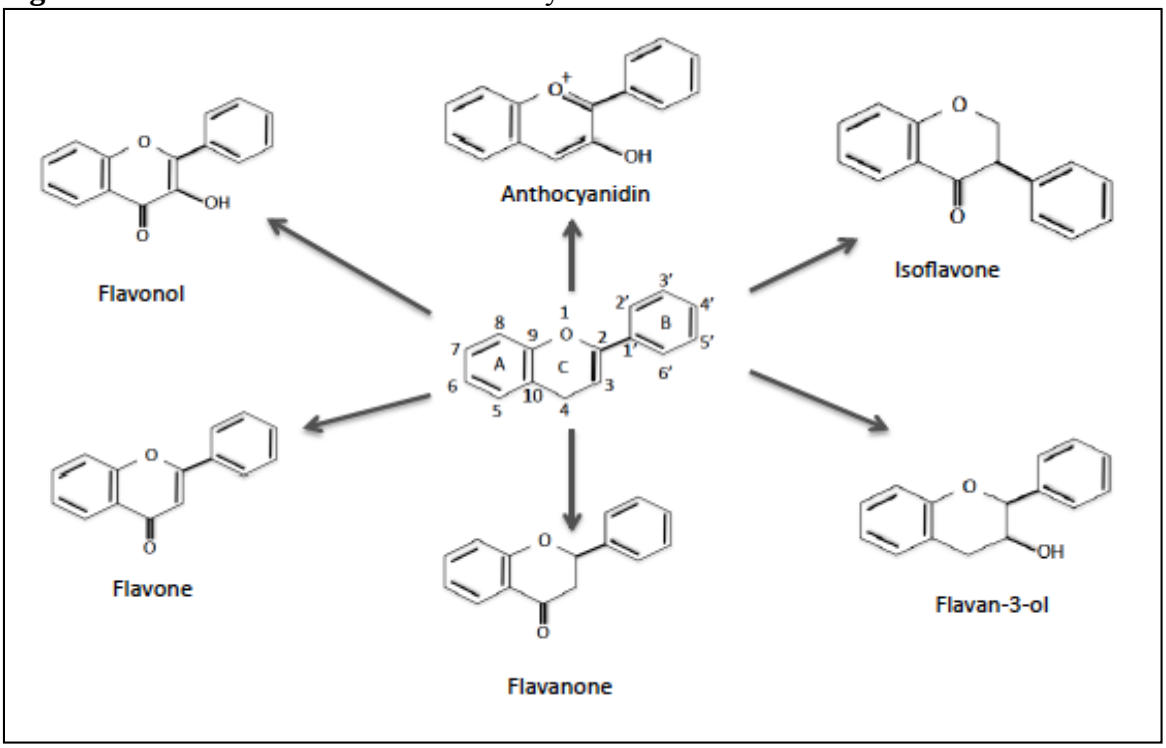
Overview of flavonoids and flavonoid subclasses

Flavonoids are non-nutrient, bioactive, polyphenolic secondary metabolites occurring naturally in vascular plants. In plants, flavonoids are thought to protect against UV radiation and microbial infection, and act as cell-signaling molecules. Flavonoids are one of the major classes of polyphenols found in the human diet, along with stilbenes, phenolic acids and lignans.[37,38] Flavonoids are metabolized but are not synthesized by animals.[39] Unlike traditional micronutrients, such as vitamins and trace elements, flavonoids are not known to be essential for short-term wellbeing and do not have an associated deficiency syndrome. Adults in the U.S. consume roughly 200 mg of flavonoids daily with the most important food sources of flavonoids in the U.S. being tea, citrus fruits, berries, wine, dark green and deep yellow vegetables, and apples.[34] In nationally representative studies, total flavonoid intake was found to increase with age until 70 years old, alcohol consumption, educational status, and household income.[34,35] In addition, non-Hispanic whites tend to consume more total flavonoids than minority populations, though among subclasses, flavanone (predominantly from citrus fruits and juices) intake is lowest among non-Hispanic whites.[35] The major dietary sources of flavonoids and total flavonoid intake remained stable between 1999-2002 (201.0 mg/day) and 2007-2010 (200.1 mg/day) though, the per capita consumption of those food sources changed. For example, per capita consumption of citrus juices declined by 29%, while there was an increase in per capita consumption of berries, wine and tea, though all of these foods remained as a top dietary source of flavonoids. Tea remained the top dietary source of flavonoids.[34]

Flavonoid subclasses

Individual flavonoids can be assigned to subclasses as follows; flavonols, flavones, flavan-3-ols, flavanones, anthocyanidins, proanthocyanidins and isoflavones. The structures for the six monomer subclasses, flavonols, flavones, flavanones, flavan-3-ols, anthocyanidins, and isoflavones are shown in **figure 2.1** and are characterized by two aromatic rings connected by a three-carbon bridge. The seventh class, proanthocyanidins, is comprised of polymers of flavan-3-ols with a wide variety of different structures. The pattern of flavonoids synthesized by plants tends to be characteristic of botanical families.[40] Some plants may be particularly high in one class, such as citrus fruits and flavanones or soy and isoflavones, however, no food contains only one class of flavonoids and it is possible that the complementary nature of these compounds result in protective effects.

Figure 2.1 General structures of commonly studied flavonoid subclass monomers



Flavonols

Flavonols are found in a wide variety of fruits and vegetables, like apples, onions, broccoli and onions.[41] The four most commonly studied flavonols are quercetin, isorhamnetin, kaempferol, and myricetin. Within the flavonol subclass, quercetin is found in the diet in the greatest abundance, representing approximately 75% of total daily flavonol intake.[35] The primary source of flavonol in the U.S. diet from 1999-2002 and 2007-2010 was tea (36.9%).[34] Flavonols are the only flavonoid subclass that does not differ between green tea and black tea, as opposed to monomeric flavan-3-ols, which are the flavonoid subclass most commonly associated with tea.[41] The top 10 sources of flavonol intake between 1999-2002 and 2007-2010 are shown in **table 2.1**.

Table 2.1 Top 10 dietary sources of flavonols among U.S. adults in National Health and Nutrition Examination Survey between 1999-2002 and 2007-2010[1]

1999-2002				2007-2010		
Rank	Food	Average intake (mg/d)	%	Food	Average intake (mg/d)	%
1	Tea	6.1	39.5	Tea	5.9	37.1
2	Other vegetables[2]	2.1	13.4	Other vegetables[2]	2.1	13.2
3	Alcoholic beverages[3]	1.3	8.6	Mixed dishes w/meat	1.5	9.3
4	Mixed dishes w/meat	1.3	8.4	Alcoholic beverages[3]	1.1	7.2
5	Dark green vegetables	0.9	5.5	Apple	0.9	5.4
6	Grain mixture, soups	0.7	4.3	Grain mixture, soups	0.8	5.3
7	Apple	0.5	3.5	Dark green vegetables	0.8	4.8
8	Legumes	0.4	2.6	Lettuce, salads	0.4	2.7
9	Lettuce, salads	0.4	2.4	Legumes	0.3	1.8
10	Tomatoes	0.3	2.0	Berries	0.3	1.8

1. Adapted from Kim et al.[34]

2. Raw and cooked vegetables and their mixtures other than potatoes, dark green and deep yellow vegetables, tomatoes, lettuce, green beans, corn, peas, and lima beans.

3. Beer, ale, liquers, cocktails, other mixed drinks, and distilled liquors excluding wine

Flavones

In general, the dietary intake of flavones is relatively small, representing less than 1% of the total daily intake of flavonoids. The two commonly studied flavones in epidemiologic literature are luteolin and apigenin. Herbs (oregano, parsley, thyme and cilantro), peppers and celery are particularly rich sources of flavonoids and are often incorporated into mixed dishes.[41] The most common dietary sources of flavones are shown in **table 2.2**. Dietary sources and per capita average consumption have remained stable over time.

Table 2.2 Top 10 dietary sources of flavones among U.S. adults in National Health and Nutrition Examination Survey between 1999-2002 and 2007-2010[1]

1999-2002				2007-2010		
Rank	Food	Average intake (mg/d)	%	Food	Average intake (mg/d)	%
1	Grain mixture, soups	0.3	34.8	Grain mixture, soups	0.4	37.3
2	Other vegetables[2]	0.3	29.4	Other vegetables[2]	0.4	29.1
3	Mixed dishes w/meat	0.1	10.9	Mixed dishes w/meat	0.1	12.2
4	Other fruits[3]	0.1	7.3	Other fruits[3]	0.1	4.9
5	Dark green vegetables	0.0	4.4	Dark green vegetables	0.0	2.8
6	Lettuce, salads	0.0	2.9	Lettuce, salads	0.0	2.6
7	Potatoes	0.0	2.7	Legumes	0.0	2.0
8	Citrus fruits	0.0	1.6	Apples	0.0	1.9
9	Apple	0.0	1.4	Wines	0.0	1.4
10	Legumes	0.0	1.0	Potatoes	0.0	1.3

1. Adapted from Kim et al.[34]

2. Raw and cooked vegetables and their mixtures other than potatoes, dark green and deep yellow vegetables, tomatoes, lettuce, green beans, corn, peas, and lima beans.

3. Fruits and their mixtures other than citrus fruits, dried fruit, apples, banana, and berries

Flavan-3-ols

Flavan-3-ols are the predominant source of dietary flavonoids in the United States, with an estimated daily intake of 158 mg daily.[34] Green and black teas contain high concentrations of flavan-3-ols, with at least 7 different flavan-3-ols in green tea alone. Besides tea, other rich sources of flavan-3-ols include apples, blueberries, and cocoa.[42] Flavan-3-ols represent the monomeric subunit from which proanthocyanidins are constructed.[43] **Table 2.3** shows the predominant dietary sources of flavan-3-ols in the U.S. adult diet.

Table 2.3 Top 10 dietary sources of flavan-3-ols among U.S. adults in National Health and Nutrition Examination Survey between 1999-2002 and 2007-2010[1]

1999-2002				2007-2010		
Rank	Food	Average intake (mg/d)	%	Food	Average intake (mg/d)	%
1	Tea	158.3	96.3	Tea	150.0	94.8
2	Apple	1.2	0.8	Apple	2.0	1.2
3	Banana	1.1	0.7	Banana	1.2	0.8
4	Alcoholic beverages[2]	0.9	0.5	Wines	1.1	0.7
5	Other fruits[3]	0.7	0.4	Other fruits[3]	0.9	0.6
6	Fruit juice, not citrus	0.5	0.3	Alcoholic beverages[2]	0.7	0.5
7	Cakes, cookies, pies	0.4	0.2	Fruit juice, not citrus	0.5	0.3
8	Milk and milk drinks	0.3	0.2	Berries	0.4	0.3
9	Coffee	0.3	0.2	Cakes, cookies, pies	0.3	0.2
10	Berries	0.2	0.1	Milk and milk drinks	0.3	0.2

1. Adapted from Kim et al.[34]

2. Beer, ale, liqueurs, cocktails, other mixed drinks, and distilled liquors, excluding wine.

3. Fruits and their mixtures other than citrus fruits, dried fruit, apples, banana, and berries

Flavanones

Flavanones are a subclass of flavonoids that are primarily found in citrus fruits and in smaller quantities in red wine and tomatoes.[42] In the United States, between 2007-2010, U.S. adults consumed on average 12.2 mg of flavanones daily, mainly from naringenin, hesperetin and eriodictyol, which are also the most studied chemicals in the

flavanone subclass.[35] Flavanones are also the second highest contributor of total dietary flavonoids in the United States, with the majority of flavanones coming from intake of citrus fruits. The main dietary sources of flavanones remain largely unchanged, however mean flavanone intake decreased by 7.7% between the period 1999-2002 and 2007-2010, see **table 2.4**[34]

Table 2.4 Top 10 dietary sources of flavanones among U.S. adults in National Health and Nutrition Examination Survey between 1999-2002 and 2007- 2010[1]

1999-2002				2007-2010		
Rank	Food	Average intake (mg/d)	%	Food	Average intake (mg/d)	%
1	Citrus fruit juices	9.6	72.7	Citrus fruit juices	7.8	63.7
2	Citrus fruit	2.9	21.7	Citrus fruit	3.3	27.0
3	Fruit drinks	0.2	1.4	Wines	0.3	2.5
4	Fruit juice, not citrus	0.1	1.1	Fruit drinks	0.3	2.1
5	Alcoholic beverages[2]	0.1	0.9	Alcoholic beverages[2]	0.2	1.5
6	Tomatoes	0.1	0.8	Tomatoes	0.1	0.9
7	Other fruits	0.0	0.3	Fruit juice, not citrus	0.1	0.8
8	Wines	0.0	0.2	Fish and shellfish	0.0	0.3
9	Fish and shellfish	0.0	0.2	Other fruits	0.0	0.3
10	Carbonated soft drinks	0.0	0.2	Mixed dishes w/meat	0.0	0.2

1. Adapted from Kim et al.[34]

2. Beer, ale, liqueurs, cocktails, other mixed drinks, and distilled liquors, excluding wine.

Anthocyanidins

Anthocyanidins are water-soluble plant pigments that give blue, purple and red colors to many fruits, vegetables and other plant tissues, like flower petals. Evidence of such pigmentation is seen in the most common U.S. food sources of anthocyanidins, which include wine and berries. Commonly studied anthocyanidins include cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin. Particularly rich sources of anthocyanidins include berries, red cabbage and plums. The potential to consume large

quantities is high considering that ½ cup of blueberries (75 grams) contains 100-130 mg of anthocyanidins.[42] However, the mean daily intake of anthocyanidins in U.S. adults between 2007-2010 was estimated to be only 11.5 mg/day. While the most common dietary sources of anthocyanidins remained the same between 1999-2002 and 2007-2010 (see Table 2.5), there was a 71.5% increase in mean anthocyanidin intake and wine became a more important contributor of anthocyanidin intake.[34]

Table 2.5 Top 10 dietary sources of anthocyanidins among U.S. adults in National Health and Nutrition Examination Survey between 1999-2002 and 2007-2010[1]

1999-2002				2007-2010		
Rank	Food	Average intake (mg/d)	%	Food	Average intake (mg/d)	%
1	Berries	2.1	31.1	Berries	4.4	38.5
2	Banana[2]	1.3	20.0	Wines	2.1	18.0
3	Other vegetables[3]	1.1	17.1	Bananas[2]	1.5	12.9
4	Cakes, cookies, pies	0.4	6.7	Other vegetables[3]	1.1	9.4
5	Other fruits[4]	0.4	6.0	Other fruits[4]	0.7	6.2
6	Wines	0.3	4.7	Apple	0.3	2.7
7	Legumes	0.2	2.8	Cakes, cookies, pies	0.3	2.7
8	Apple	0.2	2.8	Milk and milk drinks	0.3	2.7
9	Milk and milk drinks	0.1	1.6	Legumes	0.2	1.5
10	Pancakes, waffles	0.1	1.5	Fruit juice, not citrus	0.2	1.5

1. Adapted from Kim et al.[34]

2. In the newest version of USDA flavonoid database, anthocyanidin values changed to zero

3. Raw and cooked vegetables and their mixtures other than potatoes, dark green and deep yellow vegetables, tomatoes, lettuce, green beans, corn, peas, and lima beans.

4. Fruits and their mixtures other than citrus fruits, dried fruit, apples, banana, and berries

Isoflavones

The most commonly studied isoflavones in epidemiologic studies are daidzein, genistein and glycetien. Isoflavones are the most bioavailable flavonoid though they are also consumed in the small quantities by adults in the US.[34] Isoflavone intake is much

higher in Asian countries where the consumption of soy is higher, around 29 mg/day.[44] Soybeans and soy products, such as tofu and soymilk, are generally considered to be the primary dietary sources of isoflavones. However, soy products, such as soy flour and soy protein isolates, are increasingly used as ingredients in a variety of foods available to consumers. Isoflavones are unique in that their structure resembles that of estrogen. Isoflavones have weak estrogenic activity, which has been proposed as a biological mechanism by which soy products may reduce cardiovascular disease.[45] **Table 2.6** shows the predominant sources of isoflavone intake in U.S. adults.

Table 2.6 Top 10 dietary sources of isoflavones among U.S. adults in National Health and Nutrition Examination Survey between 1999-2002 and 2007-2010[1]

1999-2002				2007-2010		
Rank	Food	Average intake (mg/d)	%	Food	Average intake (mg/d)	%
1	Legumes	0.4	45.2	Legumes	0.4	41.6
2	Coffee	0.1	9.6	Mixed dishes w/meat	0.1	11.1
3	Mixed dishes w/meat	0.1	9.1	Coffee	0.1	9.3
4	Cakes, cookies, pies	0.1	7.1	Cakes, cookies, pies	0.1	7.9
5	Sausages, organ meats[2]	0.1	7.0	Sausages, organ meats[2]	0.1	7.0
6	Yeast breads, rolls	0.0	4.8	Citrus fruit juices	0.0	3.7
7	Citrus fruit juices	0.0	4.3	Yeast breads, rolls	0.0	3.5
8	Grain mixtures, soups	0.0	1.9	Milk and milk drinks	0.0	3.2
9	Eggs	0.0	1.6	Grain mixtures, soups	0.0	2.8
10	Other vegetables[3]	0.0	1.6	Other vegetables[3]	0.0	2.3

1. Adapted from Kim et al.[34]

2. Organ meats, sausages and lunchmeats, and meat spreads

3. Raw and cooked vegetables and their mixtures other than potatoes, dark green and deep yellow vegetables, tomatoes, lettuce, green beans, corn, peas, and lima beans.

Proanthocyanidins (PA)

Proanthocyanidins are polymeric forms of flavan-3-ols. Major food sources in the US include tea, wine and legumes. Other foods with high proanthocyanidin concentrations are cocoa, berries, plums and many nuts. The most recent estimates of U.S. proanthocyanidin intake are based on the National Health and Nutrition Examination Survey III (1988-1994) 24-hour dietary recall.[35] The mean proanthocyanidin intake for U.S. adults was 98 mg/day and the top five dietary sources of proanthocyanidin were, in order, wines (24.9%), legumes (18.2%), tea (15.5%), strawberries (12.9%) and plums (10.3%).

Development of the Provisional Flavonoid Addendum

One of the challenges of flavonoid research is the absence of a comprehensive database of flavonoid values for foods and beverages. Several U.S. Department of Agriculture (USDA) special interest databases containing flavonoid values for a limited number of foods have been used in the majority of flavonoid research to date.[46-49] These flavonoid values are analytic values obtained from literature review and while they meet USDA data quality standards[50], many food items do not have recorded analytic flavonoid values. Food scientists often focus on analyzing just the predominant flavonoid compounds in a food, leaving flavonoid profiles incomplete. Also missing from databases are values for multi-ingredient foods like fruit yogurts, many cooked or processed foods, and foods unlikely to contain flavonoids, such as meats and dairy. The lack of a comprehensive food composition database leads to potential measurement error in flavonoid estimates. To address these issues, the USDA Nutrient Data Laboratory developed the Expanded Flavonoid Database for the Assessment of Dietary Intakes (FDB-EXP), which vastly increased available flavonoid values.[41] The FDB-EXP was then shared with the USDA

Food Surveys Research Group to develop flavonoid values for over 7,000 foods in the Food and Nutrient Database for Dietary Studies (FNDDS), 4.1, which can be used to estimate flavonoid intakes for the U.S. population.[25] A detailed discussion about the USDA databases and the development of the flavonoid databases for use in this study can be found in Chapter 3.

Flavonoids and proposed pleiotropic effects in cardiovascular disease

Atherosclerosis, a major cause of cardiovascular disease, is characterized by chronic inflammatory and oxidative stress. Early stages of atherosclerosis involve vascular inflammation and endothelial dysfunction.[51] Oxidized low-density lipoprotein (ox-LDL) within the arterial intimal layer and concomitant oxidative stress, trigger vascular endothelial cells to express intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).[52] These adhesion molecules allow monocytes to attach to the endothelial surface, migrate into the arterial intima and differentiate into macrophages.[53] Differentiated macrophages expressing scavenger receptors engulf ox-LDL, become foam cells and lead to fatty streak formation.[54] Molecules produced by monocytes, macrophages and arterial cells also lead to sustained inflammation and proliferation of vascular smooth muscle cells.[51,53] Proliferative smooth muscle cells release fibrinogenic mediators and build a dense extracellular matrix around foam cells and monocytes leading to fibrous plaque formation.[55] Atherosclerosis is a complex cycle of oxidation and inflammation with multiple potential biological targets at which nutrients, such as flavonoids, can act.

Flavonoids and their health benefits in capillary wall stability, were described as early as 1936.[56,57] In the 1980s interest in flavonoids as antioxidants grew[58,59] as well as

interest in other potential cardioprotective effects, such as modulation of platelet function, interference with arachidonic acid metabolism [60], and modulation of lipid metabolism.[61] Until recently, direct antioxidant activity (free radical scavenging) was considered to be among the main cardioprotective effects of flavonoids, though scientific evidence no longer firmly supports the theory of direct antioxidant activity.[62]

Direct antioxidant effects of flavonoids are unlikely because flavonoids have limited bioavailability. Concentrations in systemic circulation and tissue concentration are also lower than other endogenous and exogenous antioxidants.[62] More recent evidence suggests that flavonoids regulate oxidant status indirectly. Flavonoids and their metabolites inhibit radical-producing enzymes, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase, resulting in reduced free radical production.[58,59,63] Flavonoids also appear to preserve the activity of paraoxonase-1,[64] a major anti-atherosclerotic enzyme found in HDL, known to protect low density lipoprotein (LDL) from oxidation.[65] Furthermore, flavonoids may interfere with intracellular redox signaling cascades and modulate the activity of redox-sensitive transcription factors.[66] The indirect antioxidant activity of flavonoids is intertwined with the anti-inflammatory activity of flavonoids. For example, flavonoids modulate the activity of neutrophils, important cellular mediators of inflammation, which produce superoxide anion when stimulated.[67]

Study of the anti-inflammatory properties of flavonoids has also been a long-standing investigational route to explain the cardioprotective benefits of flavonoids. In addition to modulation of neutrophil function, flavonoids target other anti-inflammatory pathways. Flavonoids influence transcription factors that direct the inflammatory process,

such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and its modulator poly (ADP-ribose) polymerase-1 (PARP-1).[68,69] The NF-κB pathway is a proinflammatory signaling pathway leading to the expression of proinflammatory genes for cytokines, chemokines and adhesion molecules, and has been implicated in the proliferation of vascular smooth muscle cells. In general, PARP-1 is activated in situations of excessive DNA damage stimulating gene repair, apoptosis or in extreme cases, leads to necrosis. The ability of flavonoids to interfere with arachidonic acid metabolism may also reduce inflammation.[60] Arachidonic acid is an omega-6 polyunsaturated fatty acid, found in cell membranes, that is the precursor for the synthesis of prostaglandins and thromboxane A_2, collectively known as prostanoids. Prostanoids are produced in trivial amounts under physiological conditions, but levels are increased in inflammatory states and if not properly balanced, lead to increased platelet aggregation, smooth muscle contraction and proliferation, and activation of endothelial inflammatory responses.[70] Finally, flavonoids and their metabolites likely inhibit adhesion molecule expression on endothelial surfaces.[71,72] Soluble vascular adhesion molecule-1 (sVCAM-1) has long been implicated as a driver of early atherosclerosis by binding monocytes that later cross the endothelium into the intimal layer and ultimately become activated foam cells secreting inflammatory cytokines. In summary, flavonoids and their metabolites exert anti-inflammatory effects by multiple mechanisms, including inhibition of NF-κB, PARP-1, adhesion molecules as well as interference with arachidonic acid metabolism and modulation of neutrophil function.

In addition to antioxidant and anti-inflammatory activity, several other cardioprotective effects of flavonoids have been described. Among these are weak

estrogenic effects[73-75], modulation of lipid metabolism[61] and platelet function[60], augmentation of nitric oxide status and modulation of nitric oxide synthase activity/expression[76,77], and potentially epigenetic modulation.[78] The intrinsic pleiotropic effects of flavonoids render them suitable to preventing or mitigating the onset and course of complex chronic diseases such as cardiovascular disease. **Figure 2.2** summarizes the potential cardioprotective effects of flavonoids.

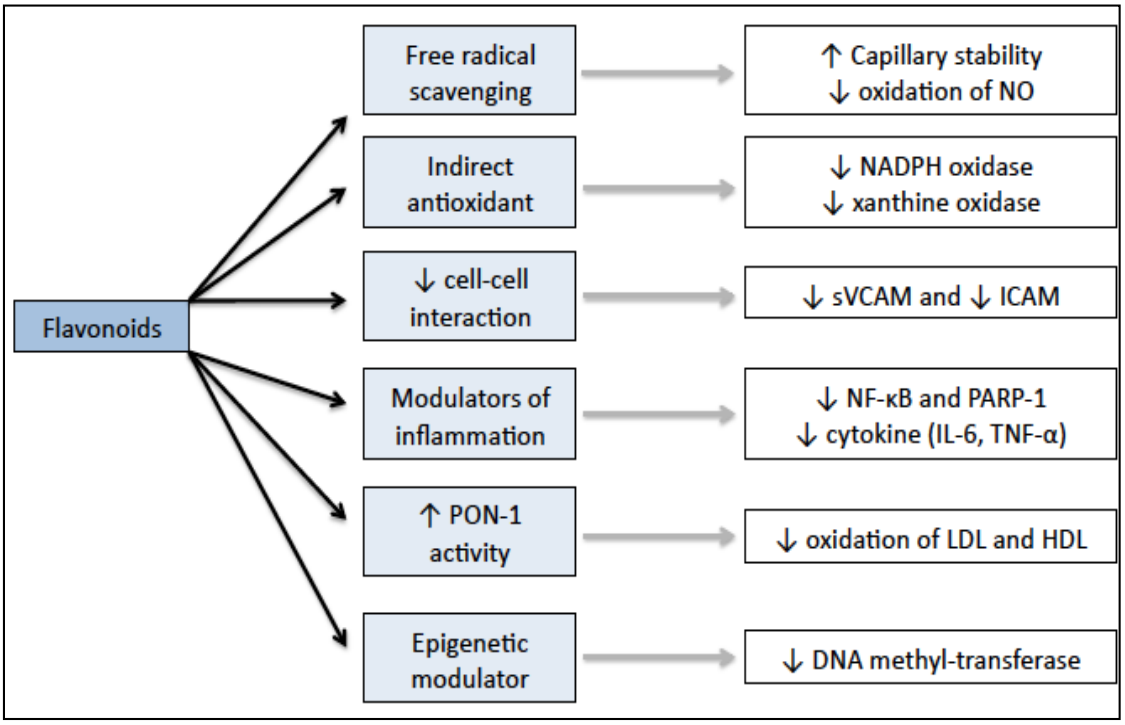


Figure 2.2. Select potential cardioprotective mechanisms of flavonoids, arranged chronologically, from top to bottom, by discovery starting with free radical scavenging. HDL, high-density lipoprotein; ICAM, intercellular adhesion molecule; IL-6, interleukin-6; LDL, low-density lipoprotein; NADPH, nicotinamide adenine dinucleotide phosphate ; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PARP-1, poly(ADP-ribose) polymerase-1 ; PON-1, paraoxonase-1 ; sVCAM, soluble vascular adhesion molecule-1; TNF-α, tumor necrosis factor – alpha.

Flavonoids and Pleiotropic effects in Neurodegenerative Disease

The proposed beneficial properties of flavonoids are not limited to cardiovascular disease; rather they extend to other complex chronic diseases like neurodegenerative conditions, such as mild cognitive impairment (MCI) and Alzheimer's dementia (AD). The precise underlying mechanisms responsible for neurodegenerative diseases like mild cognitive impairment and dementia are not clear. Several processes are potential candidates, including increases in oxidative stress, neuroinflammation, loss of synaptic plasticity, carrying the apolipoprotein E4 (APOE4) genotype, and deposition of aggregated proteins like amyloid plaques and neurofibrillary tangles.[79] Traditional cardiovascular risk factors have also been associated with increased risk of cognitive decline.[80] In fact, CVD risk factors present in midlife are associated with cognitive decline in later years.[5,6]

A structurally unique endothelial barrier different from other vascular barriers protects the brain. Molecules that can traverse vascular barriers throughout the body are not necessarily able to cross the blood brain barrier (BBB). For flavonoids to be active within the central nervous system, they need to be able to cross the BBB and evidence suggests that this is the case, at least, for flavonols[81], flavanones[82,83], flavan-3-ols[84] and anthocyanidins.[85] Given the roles of neuroinflammation, oxidative stress and deposition of aggregated proteins as potential pathophysiologic mechanisms leading to cognitive decline, the pleiotropic effects of flavonoids make them potential neuroprotective candidates. Causes of neurodegeneration and possible neuroprotective mechanisms of flavonoids are summarized in **Figure 2.3**.

In most cases, neuroinflammation serves as beneficial biologic process that protects the central nervous system against injury and infection.[86] However, sustained and unregulated neuroinflammation leads to permanent damage, such as impeding the efficiency of long-term potentiation, an important neurophysiologic process needed for memory formation, and potentiating the effects of amyloid proteins, seen in AD and MCI.[87-89] Flavonoids appear to exert neuroprotective effects by suppressing activation of microglia in the brain, thereby (i) inhibiting the release of inflammatory cytokines like TNF-alpha, interleukin-1beta and C-reactive protein, (ii) inhibiting inducible nitric oxide synthase (iNOS) and (iii) suppressing activation of the transcription factor NF-KB.[90]

In addition to vascular and anti-inflammatory effects, flavonoids may stimulate synaptic plasticity. Long-term potentiation (LTP), a long-lasting increase in synaptic strength, is related to the formation of long-term memories in which changes to neural pathways take place for storage of information that can be recalled after weeks to years.[91] Long-term memory formation depends on a temporally limited phase of RNA and protein synthesis, which is controlled in the context of LTP. In animal models, flavonoids were observed to activate the protein synthetic pathways considered critical in the induction of long-lasting changes in memory.[92,93] For example, aged animals fed a variety of flavonoids including anthocyanidins, flavones, flavan-3-ols and isoflavones, in the form of foods or extracts, have demonstrated inhibition of microglial overactivation[94], improvements in memory tasks and reduced ischemia-induced neuronal damage in the hippocampus.[95-97]

Finally, the beneficial vascular effects of flavonoids may play a role in neurogenesis. Endothelial cells that line neural vasculature are a part of the neural stem

cell niche and release soluble factors that participate in the regulation of neural stem cell proliferation and differentiation.[98] Preservation of vascular function could help preserve endothelial progenitor cell recruitment and subsequent neurogenesis. For example, increased neuronal spine density and proliferation of precursor cells in the hippocampi of mice and rats have been measured following green tea flavan-3-ol and blueberry supplementation, respectively.[99,100] Though this evidence is limited to animal models, if true in humans, then flavonoids may protect against neurodegeneration and cognitive decline by repopulation of hippocampal neurons.

Evidence that flavonoids are able to cross the BBB, localize in brain tissue combined with in situ evidence of anti-inflammatory activity, promotion of long-term potentiation, neurogenesis and improved cognitive performance in animal models suggests that flavonoids are potential candidates as neuroprotectants.

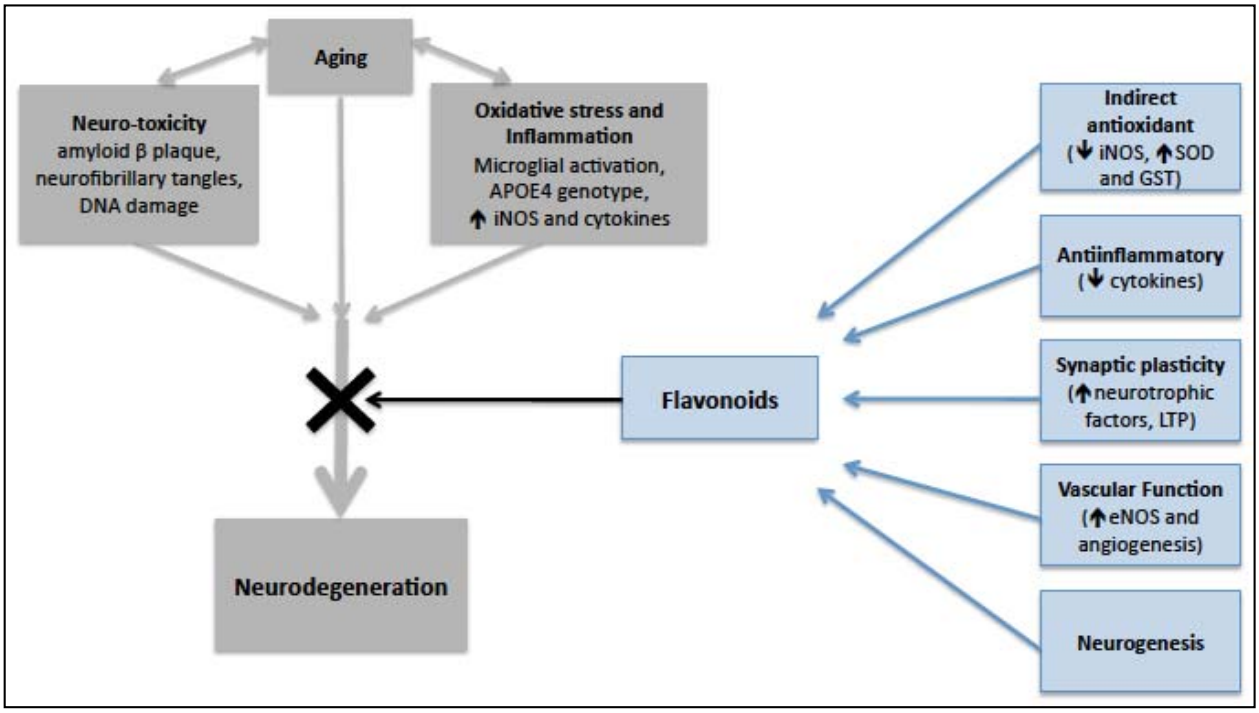


Figure 2.3 Summary of causes of neurodegenerative disease and potential neuroprotective mechanisms of flavonoids.[15]

Flavonoids and cardiovascular risk factors in humans

Hypertension

Evidence describing the association between habitual flavonoid intake and cardiovascular risk factors is relatively sparse. Hypertension is an important risk factor for the development of cardiovascular disease, including stroke and CHD. Globally, in the year 2001, roughly 54% of stroke and 47% of ischemic heart disease was attributable to hypertension.[101] The association between blood pressure and ischemic heart disease and stroke is linear with the risk of disease roughly halved for every 20 mmHg increase in systolic blood pressure and 10 mmHg in diastolic blood pressure.[102] A variety of flavonoid compounds and subclasses have been associated blood pressure lowering effects, including flavonols, flavanones, flavan-3-ols from tea and anthocyanidins.[103] For example, anthocyanidins (typically found in red and blue colored fruits and vegetables) and flavones (often found in aromatic herbs, hot peppers, and celery) were associated with lower arterial stiffness as measured by central blood pressure and pulse-wave velocity (PWV), in a cross-sectional study of twin women.[21] Greater habitual intake of anthocyanidins, flavones (particularly apigenin) and flavan-3-ols (particularly those from green tea) were also associated with a 5-8% reduced relative risk of incident hypertension in men and women.[22]

Inflammation

Inflammation is implicated as one of the major pathophysiologic processes underlying a variety of chronic diseases, including atherosclerosis. Flavonoids are associated with a wide range of inflammatory markers and higher intake of anthocyanidins is associated with lower C-reactive protein[104,105] and fibrinogen[105], both

acute phase reactants. Habitual flavonol intake has also been inversely associated with circulating cytokine levels, including IL-6, IL-8, and TNF-alpha, [105] as well as circulating levels of sVCAM.[106]

Obesity

Abdominal obesity is a recognized risk factor for cardiovascular disease with a 1 cm increase in waist circumference associated with a 2% increase in risk of incident CVD events and a 0.01 unit increase in waist-to-height ratio associated with a 5% increase in risk.[107] Most studies of flavonoids and weight have been weight loss trials examining supplemental green tea flavan-3-ols. These studies mainly were randomized controlled trials of short duration, typically 12-13 weeks long, which may be insufficient follow-up time to assess changes in weight. In metaanalyses, there was a small but non-significant weight loss in overweight and obese adults.[108] A few epidemiologic studies have assessed long-term habitual flavonoid intake and measures of obesity. Results from three prospective cohort studies suggest that habitual flavonoid intake may improve weight maintenance over time. Over fourteen years, high flavonol/flavones and high catechin (flavan-3-ol) intake were associated with lower increases in BMI in women, though not in men. [109] More recently greater intake of anthocyanidins, proanthocyanidins and flavonols was inversely associated with weight change over 24 years of follow up, in both men and women.[110] These results are supported by the finding that increased intake of fruits that are rich in flavonoids, including blueberries, apples, pears, prunes, strawberries and grapes are also associated with less weight gain over 24 years.[111]

Insulin sensitivity/Type 2 Diabetes

Greater adherence to plant based diets that are associated with reduced incidence of cardiovascular events, such as the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) diet are also associated with reduced fasting glucose and fasting insulin levels, respectively. [112,113] The relationship between fruit and vegetable intake and the risk of type 2 diabetes (T2DM) is not as clear. In metaanalyses, only increasing daily intake of green leafy vegetables was associated with reduced risk of T2DM (pooled HR: 0.8, 95% CI 0.77, 0.97, p=0.01).[114] Mechanistic studies suggest biologic pathways by which flavonoids may support insulin sensitivity. In animal models of T2DM, anthocyanidins significantly reduced blood glucose concentrations and increased insulin sensitivity in rats given a glucose tolerance test. Theses changes were accompanied by an increase in gene expression of the glucose transporter GLUT4 in adipose tissue and decrease in retinol binding protein 4 which led to suppression of gluconeogenesis and improvements in blood glucose levels.[115] In human studies, blueberry supplementation led to improved insulin sensitivity[116] and reduced fasting plasma glucose.[117] In contrast, trials using strawberry powder did not result in improved glycemia, though there were improvements in LDL and total cholesterol.[118] Different results might be due to different distributions of anthocyanidins in blueberries and strawberries. Given biologically plausible mechanisms by which flavonoids might beneficially affect glycemia, several prospective cohort studies have examined the association between habitual flavonoid intake and risk of T2DM.[104,119-121] In metaanalysis, there was a small, though statistically significant association, such that those with the highest intake of total flavonoids compared to the lowest experienced a 9% lower relative risk of T2DM.[122]

Habitual dietary flavonoid intake has been examined in relation to a variety of cardiovascular risk factors. Among these, there is evidence that greater intake of certain flavonoid subclasses, especially anthocyanidins, is associated with reduced acute phase reactants in the setting of inflammation, reduced blood pressure, lower waist circumference and small decrease in the risk of type 2 diabetes. As these conditions are also associated with increased risk of incident cardiovascular disease and cognitive impairment and dementia, these may be along the causal pathway between flavonoid intake and cardiovascular and cognitive health.

Epidemiologic studies of flavonoids and cardiovascular disease

Participants

Twenty-four studies examining flavonoid intake and various coronary heart disease or stroke endpoints have been published, using 15 different prospective European or U.S. cohorts since 1993.[13,16,18-20] Of these, seven cohorts were from the U.S., including the Nurses' Health Study (NHS), Nurses' Health Study II (NHS II), Health Professionals Follow-Up study (HPFS), the Iowa Women's Health Study, the Women's Health Study (WHS), the Framingham Offspring and the Cancer Prevention Study II (CPS II) cohorts. Five of the 15 cohorts included both men and women of which 2 were in the U.S. (CPS II and Framingham Offspring). Four U.S. studies included only women (NHS, NHS II, Iowa Women's Health Study, and WHS) and one included only men (HPFS). In the U.S. none of these studies were diverse in terms of race/ethnicity or geography and three of the seven included only health professionals (NHS, NHS II and HPFS).

Flavonoid Assessment

A variety of different dietary assessment tools were used in these studies, including food frequency questionnaires of varying lengths, diet histories and in one case, a diet checklist with a dietitian interview and food frequency questionnaire. The food flavonoid composition tables used for these studies also varied widely. European studies cited different versions of seven different flavonoid databases including those developed by Hertog et al. containing flavones and flavonols[123-126], Arts et al. containing flavan-3-ols[127,128], Boker et al. containing isoflavones[129], Hakkinen et al. containing flavonols from berries[130], Starke et al. and Wildanger et al. containing flavonols from fruits[131,132], and Mattila et al. with a variety of flavonoids.[133] Studies from U.S. cohorts cited various versions of the United States Department of Agriculture flavonoid databases originally developed 2003.[46,47,49] Due to the variety of flavonoid databases used, there was a variety of flavonoid exposures used across studies, with earlier studies focusing on flavonols, flavones, and flavan-3-ols, while later studies included anthocyanidins, flavanones, isoflavones and proanthocyanidins. Two mortality studies included all seven subclasses[16,17], while three incidence studies included six of seven flavonoid subclasses.[18-20] No incidence studies included isoflavone intake.

Outcomes

Mortality outcomes were classified using International Classification of Disease (ICD) codes, versions 8-10. Case ascertainment for incident events differed based on the study location. Finnish cohorts identified cases in national registries followed by physician review of records. Dutch cohorts used self-report or physician-report of cases followed by physician review of records. U.S. cohorts used participant-reported cases confirmed by physician review of records.

Epidemiologic studies of flavonoids and stroke

Four studies examined stroke mortality in three cohorts (Iowa Women's Health Study, Zutphen Elderly Study and Cancer Prevention Study II).[16,17,134,135] All of the studies examined flavonol and flavone intakes and none of them found an association with stroke mortality. There were no significant associations between flavan-3-ols, flavanones, isoflavones, anthocyanidins, isoflavones or proanthocyanidins in any of the studies in which these flavonoid subclasses were examined.[16,17,136] In summary, there were no associations found between flavonoid intake and stroke mortality in four studies.

There were ten studies that examined incident stroke.[20,119,135-141] Results from studies examining flavonols and flavones, or their combination had mixed results. Flavones[138] and the combination of flavonols and flavones[140] were inversely associated with incident stroke in two studies. In contrast, three studies found no association for flavonols and flavones combined[137,139,141] and one set of studies from the same cohort found an association for only one flavonol, kaempferol, but not the others (myricetin and quercetin).[119,142] A meta-analysis of results from six cohorts[17,119,137,138,140,141] found an association significant inverse association between flavonols intake and incident stroke, comparing extreme quartiles of intake (RR: 0.80, 95% CI: 0.65, 0.98). Later studies included flavan-3-ols, flavanones, anthocyanidins, isoflavones and proanthocyanidins.[20,119,136,140] Two of three found an inverse association between flavanone intake and stroke.[20,119] There was no association between flavan-3-ols[20,119,140] , anthocyanidins[20,140], isoflavones[136], proanthocyanidins[20] and stroke in any study.

Four studies from three cohorts examined hemorrhagic and ischemic stroke separately.[20,119,137,142] Two studies found no association between flavonols or flavones

intake and thrombotic stroke.[20,137] One study found no association for quercetin, only.[142] In contrast two studies found a significant inverse association between flavanone intake and thrombotic stroke,[20,119] while one study found a nonsignificant inverse association for flavanones, flavonols and flavones combined for hemorrhagic stroke.[119] In summary, evidence supporting an association between flavonoid intake and incident stroke and stroke mortality is mixed. However, results are strongest for an association between flavonol and flavanone intakes and incident stroke.

Epidemiologic studies of flavonoids and coronary heart disease

Coronary heart disease (CHD) mortality is the most studied cardiovascular outcome in relation to flavonoid intake. Only two out of fourteen studies examined all seven subclasses of flavonoids.[16,17] Flavonols and flavones were significantly associated with reduced CHD mortality in four studies[119,134,143-147] but not in three others.[17,134,147-150] In a 2003 meta-analysis, flavonols were associated with a 20% relative reduction in risk of CHD mortality (110). Fewer studies assessed the association between other flavonoid subclass (anthocyanidins, flavan-3-ols, flavanones, isoflavones and proanthocyanidins). One study found a significant association between flavan-3-ol intake and reduced CHD mortality[135] while two did not. [17,151] Three studies examined flavanone intake and one found a significant association with reduced risk of CHD mortality.[17] Two others did not find an association.[16,119] Two studies examined anthocyanidins, isoflavones and proanthocyanidins and both found an inverse association for anthocyanidins but no association for isoflavones or proanthocyanidins.[16,17] In summary, there is some evidence

of a beneficial association between flavanone and anthocyanidin intakes and CHD mortality, but evidence is strongest for flavonol intake.

Thirteen studies have studied coronary heart disease incidence.[18,19,135,136,139,141,143-145,147-150] All thirteen studies examined flavonol and flavone intake, but only one found an inverse, though not statistically significant association with lower CHD incidence for flavonols and flavones combined.[149] In another, there was an inverse association for flavone intake, but this was also not statistically significant.[19] In a third study, only luteolin, a flavone, was associated with lower CHD incidence.[139] Three studies examined flavan-3-ol intake and found no association with incident CHD.[18,19,135] One study examined isoflavone intake as an exposure and found no association with incident CHD.[136] Only one study found a protective association between anthocyanidin intake and incident CHD in women[18], though another did not find an association.[19] Two studies have considered the relationship between proanthocyanidin intake and incident CHD and neither found an association.[18,19]

Epidemiologic studies of flavonoids and cognitive health

In spite of a growing body of experimental animal research, evidence of a protective role of flavonoids from human intervention and epidemiologic studies remains relatively sparse and unclear. Several small controlled human intervention studies have been conducted using cocoa, isoflavones, and fruit juices. Acute intervention studies have found that cocoa supplementation in healthy volunteers may improve working memory.[152-154] However, chronic cocoa interventions, lasting 30 days – 6 weeks, have no been associated with cognitive performance.[155-157] In contrast, chronic isoflavone supplementation has demonstrated mixed results with improved cognitive performance in

some studies[158,159] but not in others.[160,161] Short term supplementation with anthocyanidins from blueberry juice or supplementation with grape juice, which is high in flavonoids, has been demonstrated to improve short-term cognitive function in older individuals with existing cognitive impairment.[162,163] The available evidence about the relationship between flavonoid supplementation and cognitive function from intervention studies is unclear. Furthermore, flavonoids are not consumed as supplements, rather as part of the daily diet, which highlights the need for studies assessing habitual flavonoid intake.

A few epidemiologic studies have assessed the relationship between habitual flavonoid intake and cognitive health, using food frequency methods or diet records. A cross-sectional observational study in Norway including 2,031 men and women aged 70-74 found that diets high in flavonoid-rich foods (dark chocolate, wine and tea) were associated with better performance on cognitive tasks in a dose-dependent manner.[164] Two prospective studies within the Personnes Agées QUID (PAQUID) study in France demonstrated an inverse relationship between flavonoid intake and cognitive decline in mean and women aged 65 year or older, after 5 years[165] and 10 years[166] of follow-up. However, these studies considered only 5 flavonoid compounds over 2 subclasses, flavonols and flavones, rather than a full spectrum of flavonoid intakes. Most recently, anthocyanidin and total flavonoid intakes (which did not include isoflavones) were associated with slower progression of cognitive decline.[167] Interestingly, associations observed between flavonoid intake and cognitive performance and risk of dementia may disappear after accounting for baseline intelligence quotient.[168] Though epidemiologic evidence suggests that there is a positive association between flavonoid intake and

cognitive function, no studies have yet evaluated the association between total flavonoid intake, including all seven subclasses, and incident cognitive impairment in a racially and geographically U.S. cohort that also included both men and women.

Racial and Geographic Disparities in Cardiovascular and Cognitive Health

Racial and geographic disparities in stroke

Racial disparities in stroke have been recognized for decades. Between 1949 and 1951 the non-white to white stroke mortality ratio was 1.63 for men and 1.92 for women.[169] Overall, age-adjusted stroke mortality has decreased over time, however, stroke mortality remains higher among black Americans than any other race/ethnic group in the U.S.[170-172] Racial disparities in stroke mortality are most pronounced at younger ages where the stroke mortality rate comparing non-Hispanic blacks to white is at least three-fold higher. This disparity narrows as age increases and disappears by 85 years of age.[170,172,173] Racial disparities in stroke mortality appear to be driven, in part, by racial disparities in stroke incidence.[174] In the REGARDS study, overall age-sex-adjusted stroke incidence was 1.51 (95% CI, 1.26-1.81) times higher for non-Hispanic black participants than white participants.[174] The disparity was most pronounced in younger ages, where between ages 45-54 the incidence rate ratio for blacks compared to whites was 4.02 (95% CI, 1.23-13.11). In contrast, over the age of 85, the stroke incidence rate ratio for blacks compared to white was 0.86 (95% CI, 0.33-2.20). Case fatality and other factors may account for the greater magnitude of racial disparities in stroke mortality, compared to stroke incidence. These findings are consistent with results from the Greater Cincinnati/Northern Kentucky Stroke Study (GCNKSS), where the age-specific incidence rates were between 2 to 5-fold higher in blacks compared to whites between 35

and 64 years old.[175] About half of the racial disparity in stroke is attributable to traditional risk factors (those in the Framingham stroke risk function) and socioeconomic factors. Other factors, such as residual confounding, disproportionate impact of risk factors in blacks compared to whites and nontraditional risk factors may play a role in racial disparities in stroke.[176]

Geographic disparities in stroke were also recognized decades ago.[177,178] Those who live in the southeastern U.S. suffer from greater stroke mortality than those who live outside of the southeastern U.S. There is no standard definition for which states constitute the U.S. "stroke belt." Typically southeastern states, except for the state of Florida, are considered the "stroke belt" including, Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina and Tennessee. Within the Stroke Belt, the coastal regions of North Carolina, South Carolina and Georgia, known as the "stroke buckle" experience an even greater stroke mortality than the rest of the stroke belt.[179] Interestingly, even short-term exposure to the stroke buckle is associated with an increased risk of death due to stroke.[180] Though there is heterogeneity in stroke mortality at the county level within the stroke belt and stroke buckle[181], overall mortality in the stroke belt is roughly 20% higher than the rest of the U.S. and 40% higher in the stroke buckle as compared to the rest of the nation.[178,181,182] Geographic disparities in stroke incidence are much smaller, with an IRR for the stroke belt compared to the rest of the nation of 1.06 (95% CI, 0.87-1.29) and for the stroke buckle 1.19 (95% CI, 0.96-1.47).[174] Racial and geographic disparities in stroke incidence and mortality remain a critical public health problem that cannot be fully explained by differences in traditional stroke risk factors.

Racial disparities in heart disease

Mortality due to acute myocardial infarction (MI) and coronary heart disease (CHD) has declined since 1970.[183-186] Unfortunately, improvements in mortality have not be evenly distributed across race/ethnic groups and non-Hispanic black Americans have experienced a more shallow decline in acute CHD than their non-Hispanic white counterparts.[29,187] Racial disparities also exist in incident CHD. Black men and women were twice as likely to experience fatal CHD as their white counterparts in the REGARDS cohort (age-adjusted HR_{men}: 2.18; 95% CI, 1.55-3.06; age-adjusted HR_{women}: 1.93; 95% CI, 1.23-3.03). This disparity was accounted for by racial differences in CHD risk factors.[28] Interestingly, there were sex differences in nonfatal incident CHD, whereby black men had a lower risk of nonfatal CHD compared to white men (age-adjusted HR: 0.81, 95% CI, 0.63-1.06) whereas black women had a greater risk of nonfatal CHD compared to white women (age-adjusted HR: 1.31, 95% CI, 0.63-1.77). After adjusting for CHD risk factors, low risk for nonfatal CHD remained among black men, but excess risk for black women was attenuated. Taken together, trends in heart disease over time suggest that while progress is being made in reducing heart disease burden and mortality overall, racial disparities persist.

Racial and geographic disparities in cognitive health

Higher rates of dementia have been reported in black participants in a handful of studies. In these studies black participants had roughly twice the risk of dementia compared to white participants.[188-191] Education is an important confounder of the association between race and cognitive status. Education is thought to stimulate dendritic

growth, synapse number, and cerebral vascularization early in life, which could buffer later life neuropathology.[192,193] Studies have adjusted for education in different ways, including educational attainment and number of school years, often resulting in attenuation of excess risk among blacks, compared to whites,[189,191] but not always.[188] Residual confounding by the quality of education cannot be excluded. Longitudinal assessments of cognitive function that take into account baseline cognitive function tend to show no difference in the rate of cognitive decline by race, likely because change is less susceptible to confounding factors that are stable over time.[194] Similar to racial differences in stroke mortality, vascular risk factor burden has been hypothesized as a cause for racial differences in dementia. Hypertension has been considered as a factor explaining disparities in cognitive decline, however racial differences persist after accounting for prevalent vascular comorbidities and while continuous SBP is significantly associated with rate of cognitive change among white Americans, it is not in black Americans.[194,195] Apolipoprotein Eε4 (APOE4), is a strong risk factors for Alzheimer's dementia, however the relative risk of dementia associated with APOE4 is lower among black Americans compared to white Americans.[196-199] Presence of the APOE4 allele is also more strongly associated with rate of cognitive decline in white study participants, rather than black participants.[200] The observed disparity in dementia risk may be due to lower baseline cognitive performance. If the rate of cognitive decline does not differ by race, then those with lower baseline function may reach a testing cut point that defines dementia sooner. Cognitive test performance characteristics may differ by race. Higher false positive rates have been reported for African Americans as compared to Caucasians using a variety of psychometric tests.[201,202] Furthermore, cultural

bias may occur, as African American study participants are more likely to self-report greater cognitive impairment than their Caucasian counterparts despite a lack of difference in objective measures of health status.[203] Similarly, African American caregivers are also more likely to report greater impairment and need for health services, such as physical therapy and nursing, than Caucasian caregivers.[204] Observed racial disparities in cognitive health have been reported in many studies, though these findings may be confounded by education, socioeconomic and sociocultural factors.

Geographic disparities in incident cognitive impairment were recently documented, revealing 18% greater odds of incident cognitive impairment in those living within the southeastern U.S.[30] Geographic disparities in stroke have been well documented with a greater risk of incident stroke and stroke mortality in those living in the southeastern U.S. as compared to those who live elsewhere.[178,181] Given geographic disparities in stroke, it is plausible that geographic disparities in cognitive impairment reflect shared cerebrovascular risk factors. Self-reported stroke symptoms (including sudden painless unilateral weakness, sudden unilateral numbness, sudden loss of vision, loss of a visual field, and sudden inability to understand others or express oneself verbally or in writing) in the absence of diagnosed stroke are considered potential markers of ischemic changes in the brain. These self-reported stroke symptoms are also associated with a 24% increase in prevalent cognitive impairment.[205] MRI-identified silent brain infarcts are also associated with risk of dementia and poor cognitive functioning.[206,207] Disparities in cardiovascular risk factors are a potential target to reduce geographic disparities in cognitive impairment. Other factors that should be considered

are migration patterns, especially since exposure to the stroke buckle appears to increase stroke risk, education factors and dietary factors.

Summary

We examined the dietary sources and the potential mechanisms of the salutary effects of flavonoids. Among the pleiotropic effects of flavonoids are indirect antioxidant activity and modulation of inflammatory processes. The ability of certain flavonoids to reduce cell-cell interaction by suppressing adhesion molecule activity, in particular intercellular adhesion molecule and soluble vascular adhesion molecule-1, may slow progression of atherosclerosis. Preservation of paraoxonase-1 activity may protect LDL and HDL from oxidation, further slowing atherosclerosis progression. Cardiovascular benefits of flavonoids represent one of the pathways by which flavonoids may protect against neurodegeneration. Flavonoids' ability to cross the blood-brain barrier, promote long term potentiation and local support of cerebral blood flow may additionally promote neurologic and cognitive health. We reviewed previous studies examining the association between dietary flavonoids cardiovascular events and cognitive health. Though the results of epidemiologic studies examining the role of flavonoids in incident cardiovascular disease and cognitive outcomes is mixed, the strongest evidence is for the beneficial relationships seen for flavanones, flavonols and stroke, anthocyanidins and coronary heart disease, as well as anthocyanidins, flavones and flavonols in cognitive health. In Chapter 3, we discuss the methods used to address our research aims identified in Chapter 1. In Chapter 4 we present the association between flavonoid intake and incident stroke in a biracial, national cohort. In Chapter 5, we present the association between flavonoid intake and incident CHD events in a biracial, national cohort. In

Chapter 6 we examine the association between flavonoid intake and cognitive performance over time. Finally in Chapter 7 we bring together the findings from previous chapters and discuss future potential steps.

CHAPTER 3: METHODS

Overview

The goals for this dissertation project are to 1) evaluate the association between flavonoid intake and incident stroke and incident acute CHD in a biracial, national cohort, 2) evaluate the association between flavonoid intake and cognitive function over time in a biracial, national cohort, 3) to examine flavonoid intake by race and by geographic region, and 4) to assess for potential effect modification of observed flavonoid intake-disease associations by race and region of residence.

Exposure Assessment

Block Food Frequency Questionnaire

The theoretical basis for the food frequency method of dietary assessment is that habitual dietary intake over long periods of time is the most relevant for diet-disease relationships. Interest in long-term usual dietary intake led to the development of the food frequency questionnaire (FFQ), which is practical for large epidemiologic studies because they are easy to complete, can be self-administered, and allow for computerized processing to obtain nutrient, food and food-group variables. The food frequency method is used extensively in epidemiologic research. Rather than querying about specific meals, participants are asked to describe the usual frequency with which carefully selected, commonly consumed foods are eaten. Evidence from cognitive science research demonstrates that recall of generic memory is superior to episodic memory in dietary surveys, which is useful for food frequency methods of dietary assessment.[208-210]

The original Block Food Frequency Questionnaire (FFQ) was a self-administered questionnaire developed for epidemiologic and clinical research using data from the

Second National Health and Nutrition Examination Survey (NHANES II).[211] Food items included in the FFQ contribute at least 90% of the population total energy intake and each of 17 selected macro- and micronutrients of interest for the 11,658 adult NHANES II participants. The observed portion size for each food item included was derived from the portion size distribution obtained from NHANES II. Additional food items were included to provide an adequate estimate of dietary fiber intake and major cruciferous vegetable intake.[211] Foods suspected to be associated with health concerns were also added, including coffee, tea and artificial sweeteners. Foods important to specific ethnic or geographic subgroups were included, as well as foods whose omission could lead to misclassification of the small number of people who consume them. Foods that may be consumed as canned/frozen or fresh, when in season, were separated into individual items. For any given nutrient to be poorly represented it would have to be found in infrequently consumed foods and in foods that are not major contributors of calories, fat, protein, carbohydrates or other 14 important nutrients.

Over time, the Block FFQ has been updated as more recent data become available from National Health and Nutrition Examination Surveys.[212] The food list for the Block Questionnaire-1998 FFQ was designed based on dietary recall data collected from NHANES III. The nutrient database was developed using data from the USDA Nutrient Database for Standard Reference (SR). The Block Questionnaire-FFQ 2005 was developed using more recent data than the Block 1998 FFQ. The food list was derived from the NHANES 1999-2002 dietary recall data and the USDA Food and Nutrient Database for Dietary Studies (FNDDS), version 1.0 provided the nutrient data. The

USDA maintains a data file that allows linkage of data between the SR and the FNDDS for continuity and is released with updates of the FNDDS.

Validity of the Block FFQ

The Block FFQ has been validated in a wide variety of populations. In a population of 303 women enrolled in the Women's Health Trial Feasibility Study, dietary estimates from the FFQ were compared against a reference standard of three 4-day diet records. Correlations for most nutrients were between r = 0.5-0.6 suggesting a moderate to good ability to place individuals along a distribution of intake.[213] The Block FFQ has subsequently been demonstrated to be very similar to the Willett FFQ in its ability to predict disease outcome when compared to an interviewer-administered diet history.[214] When comparing the National Cancer Institute Diet History Questionnaire (DHQ), Willett FFQ, Block FFQ and four 24-hr recalls, the DHQ and Block FFQ were found to estimate absolute intakes better than the Willett FFQ. All three FFQs performed similarly for the purpose of assessing diet-disease relationships.[215]

Estimation of total flavonoid intake using a Block FFQ has not been validated. The Block FFQ has only been validated to estimate isoflavone intake, which is one of seven classes of flavonoids.[216,217] A method to construct a flavonoid database for use with a modified Block FFQ has been published[218] and was followed by two subsequent studies of flavonoid intake and breast cancer outcomes that demonstrated a diet-disease relationship.[219,220] Though the Block FFQ has not been validated for flavonoid intake, it has been validated for reported dietary carotenoid intake against plasma carotenoid values in low-income southern black women[221] and male non-smokers.[222] Carotenoids are primarily found in a wide variety of fruits and vegetables and are useful for the

validation of FFQs for fruit and vegetable intake because they provide good time integration, meaning that they accurately reflect dietary carotenoid intake over an extended period of time, a desirable feature for nutrients used to validate dietary assessment tools.[223]

A variety of other FFQs have been used to evaluate the association between dietary flavonoid intake and cardiovascular disease, though they were also not designed to assess flavonoid intake. National Cancer Institute DHQ, which is similar to the Block FFQ in terms of design and estimation of absolute intake of major nutrients, has demonstrated deattenuated correlations for total flavonoids, using a 24-hour dietary recall standard, of r=0.65 for women and r=0.57 for men.[224] Estimates of dietary flavonoid intakes obtained by the NCI DHQ were inversely associated with CVD mortality.[16] The Willett FFQ has been also used to estimate flavonoid intake, though it has also not been validated specifically for that purpose. However, the Willett FFQ has demonstrated very good reproducibility and validity for intake of individual food items, with deattenuated correlations between 0.70 and 0.93 for foods that are common sources of flavonoid for U.S. adults, including fruits (r=0.70), vegetables (r=0.50), tea (r=0.77), and wine (r=0.83).[225-227]

Overall, the Block FFQ is an extensively validated and utilized method to estimate reported dietary intake in epidemiologic studies. Based on existing literature, the Block FFQ performs as well as the National Cancer Institute DHQ when estimating absolute nutrient intakes, which has moderate to good correlations for flavonoid intake as compared to multiple 24-hour recalls. Though it has not been validated specifically for

flavonoid intake, existing literature and comparison to other widely used FFQs, suggests that the Block FFQ is an acceptable dietary assessment tool for dietary flavonoid intake.

USDA Databases for Flavonoid Content of Selected Foods

Databases containing information on the flavonoid content of foods have evolved in recent years to include a wider variety of flavonoids and foods, taking into account mixed dishes, especially commercially prepared foods, and the effects of cooking and processing on the flavonoid content of foods. Currently, the U.S. Department of Agriculture (USDA) maintains a variety of databases that collectively contain data on the flavonoid content for 35 important flavonoids, from 7 subclasses, in selected foods. Historically, the USDA maintained three distinct flavonoid databases. The largest of these three databases is the USDA Database for Flavonoid Content of Selected Foods (FDB), Release 3.1 [228] The FDB 3.1 contains values for 26 dietary flavonoids in five flavonoid subclasses (flavonols, flavones, flavanones, flavan-3-ols, and anthocyanidins) in 506 foods. The USDA Database for the Isoflavone Content of Selected Foods (IDB), Release 2.0 contains values for three isoflavones in 557 food items.[47] The third database is the USDA Database for the Proanthocyanidin Content of Selected Foods, which contained data for 205 foods.[46]

USDA Data Quality Standards

Data are included in USDA databases if the analytic methods by which they were obtained are acceptable. Acceptable procedures are defined as those that lead to good separation of flavonoid compounds, including column chromatography of high performance liquid chromatography (HPLC), capillary zone electrophoresis, micellar electrokinetic capillary chromatography. Excluded methods included thin layer or paper

chromatography, radioimmunoassay (RIA), pH differential methods, or only spectrophotometric quantitation.[229]

Values for data in flavonoid databases are presented in aglycone form. USDA scientists converted values that were presented in glycoside form using the molecular weight of the specific compound in order to maintain measurement consistency. Catechins and epicatechins are typically found in foods in gallic ester form and were included in the database as such, without conversion. Mean values are reported as mg/100 g of fresh weight of edible portion of food. Beverage values were adjusted by their specific gravities if reported based on liquid volume (mg/ml) in order to obtain weight (mg/100 g). Values of compounds from tea were standardized to a 1% infusion of tea (1g tea leaves/100 ml boiling water). Adjustment for brewing time was not needed because the majority of tea flavonoids are extracted within short brewing times.[128,230]

Expanded USDA Flavonoid Databases

The most recently updated flavonoid database is the Expanded Flavonoid Database for the Assessment of Dietary Intakes (FDB-EXP), which was released in September 2014.[41] This updated database, constructed by food scientists at the Nutrient Data Laboratory (NDL), provides values for roughly 2,900 foods in the National Nutrient Database for Standard Reference, Release 22 (SR22). The USDA Nutrient Data Laboratory maintains the National Nutrient Databases for Standard Reference for the purpose of disseminating food composition data. The FDB-EXP combines all analytic values from two previous USDA flavonoid databases. Analytic flavonoid values for 26 commonly occurring flavonoid compounds for 506 foods came from the USDA Database for Flavonoid Content of Selected Foods, Release 3.1 (FDB 3.1) and analytic values for

three isoflavones from 550 food items came from the USDA Database for the Isoflavone Content of Selected Foods, Release 2.0, 2008.[47,48] Values reported in the FDB-EXP are a combination of analytic and calculated values based on previously obtained analytic values.[41]

Analytic flavonoid values in the FDB 3.1 and IDB 2.0 for food groups that were expected to contain flavonoids (fruits, vegetables, spices, herbs, nuts, seeds, and legumes) and were exact matches were retained in the FDB-EXP. A variety of approaches were used to calculate previously missing values, including assigning zero values, calculating flavonoid values based on processing factors, substitution, utilization of market share data, and calculating flavonoid values for multi-ingredient foods. Animal based foods groups such as beef, pork, poultry, finfish, shellfish, and their derivatives (fats, oils, sausages, luncheon meats) were not expected to contain flavonoids and were assigned a zero value for flavonoids. Multi-component foods containing these foods were also assigned zero values if no other ingredients were expected to contain flavonoids. Calculations for processing factors were done to account for changes in moisture, such as drying to convert values for fresh herbs to dried herbs, or converting raw vegetable to cooked vegetables. Retention factors were used to calculate flavonoid values for cooked or processed foods if analytic values were not available. A factor of 85% was used for flavonols, flavan-3-ols, flavanones and flavones and 50% for anthocyanidins. Retention factors were not needed for isoflavones, as these were available in the IDB 2.0. Values for similar foods were substituted for the foods based on botanical origins, such as applying values for blackberries to mulberries. In some cases insufficient data were available for a food item, which was resolved using market share data. For example,

flavonoid values for blush wine were a composite of 50% red wine and 50% white wine. Finally, flavonoid values for multi-ingredient foods, such as soups and breakfast cereals, were calculated based on formulations developed by Nutrient Data Laboratory scientists at the USDA.[41]

The FDB-EXP contains values for 29 flavonoids in six flavonoid subclasses for 2,926 foods. Zero values were assumed for 73% of the total 87,780 values in the database. Of all values, 3% were analytic values and 24% were calculated. The FDB-EXP was shared with the USDA Food Surveys Research Group to construct the Provisional Flavonoid Addendum to the Food and Nutrient Database for Dietary Studies (FNDDS), 4.1, which was released in December 2014, and its development will be discussed below.[25] Flavonoid compounds included in the FDB-EXP and the Provisional Flavonoid Addendum are found in **table 3.1**. The FNDDS is the database that provides the nutrient values for foods and beverages reported in the dietary intake survey component of the National Health and Nutrition Examination Survey (NHANES). Proanthocyanidins were not included in the FDB-EXP.

Table 3.1 Flavonoid compounds and subclasses included in USDA Expanded Database for Assessment of Dietary Intakes (FDB-EXP) and Provisional Flavonoid Addendum[25,41]

Flavonoid subclass	Typical high content foods	Dietary flavonoids included
Flavonols	Onions, broccoli, apples	Isorhamnetin, Kaempferol, Myricetin, Quercitin
Flavones	Celery, parsley	Apigenin, Luteolin
Flavanones	Citrus fruits	Eriodictyol, Hesperitin, Naringenin
Flavan-3-ols	Apples, red wine, green tea, black tea	(+)-Catechin, (+)-Gallocatechin, (-)-Epicatechin, (-)-Epigallocatechin, (-)-Epicatechin 3-gallate, (-)-Epigallocatechin 3-gallate, theaflavin, theaflavin 3-gallate, Theaflavin 3'-gallate, Theaflavin3,3'-digallate, Thearubigins
Anthocyanidins	Red wine, blueberries, raspberries	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin
Isoflavones	Tofu, soy, legumes	Daidzein, Genistein, Glycitein

To reiterate and clarify the differences between Standard Reference databases and the FNDDS database, the SR is a nutrient database designed for dissemination of food composition data. The FDB-EXP was developed by the USDA Nutrient Data Laboratory by food scientists and provides flavonoid values for the SR, also maintained by the USDA Nutrient Data Laboratory. In contrast, the Food and Nutrient Database for Dietary Studies (FNDDS) is designed by the USDA Food Surveys Research Group, using nutrient data contained in the SR, to monitor and assess food consumption and related behaviors of the U.S. population through programs such as NHANES. The FNDDS represents foods and beverages as consumed by the U.S. population, contains no missing nutrient values and contains many additional items not in the Standard Reference.[25] The FNDDS has its own 8-digit coding system while the SR has its own unique 5-digit coding system. The USDA maintains an FNDDS-SR links file to describe the relationship between FNDDS codes and SR codes used to calculate FNDDS nutrient profiles.

Development of the Provisional Flavonoid Addendum to the FNDDS, 4.1

The Provisional Flavonoid Addendum was constructed by the USDA Food Surveys Research Group, based on the USDA's Expanded Flavonoid Database for the Assessment of Dietary Intakes (FDB-EXP). The Provisional Flavonoid Addendum is designed for the purpose of assessing flavonoid intake in the U.S. population and is an adjunct to the Food and Nutrient Database for Dietary Studies. The Provisional Flavonoid Addendum, like the FDB-EXP, does not contain estimates of proanthocyanidins. Each of the 7,174 food/beverage items in the Addendum is uniquely identified with its own 8-digit food code and flavonoid profile. This 8-digit FNDDS code can be used to link to

other dietary surveys using the same FNDDS codes. Nutrient profiles for many of the 7,174 foods were calculating using more than one of the roughly 2,900 foods from the FDB-EXP.[25]

A few assumptions made during development should be mentioned. Black and green teas have differing flavonoid profiles, however, the FNDDS, 4.1 does not differentiate between the two. Therefore a flavonoid profile for tea was calculated based on market share data assuming 84% black tea and 16% green tea. When flavonoid profiles were calculated from multiple ingredients, component foods or ingredients were omitted if they accounted for less than 5% of the weight of the food. Exceptions to this "5% rule" were made if the ingredient was likely a major contributor of flavonoids, such as soy flour, soy protein isolate or cocoa powder. Additional foods that were included even when present in small amounts were tea, onions, peppers, berries, lemons, dark leafy green vegetables and celery. Retention factors for moist-heat cooking methods in the Provisional Flavonoid Addendum were the same as those used in the FDB-EXP; 15% loss for flavonols, flavan-3-ols and flavones and 50% for anthocyanidins. No losses were assumed for dry-heat cooking methods.

USDA Database for Proanthocyanidin Content of Selected Foods

USDA scientists developed the USDA Database for the Proanthocyanidin Content of Selected Foods in collaboration with the Arkansas Children's Nutrition Center; Mars, Inc.; and Ocean Spray Cranberries, Inc.[46] This database contains proanthocyanidin content of 205 foods. Data were obtained from analyses conducted by Agricultural Research Service (ARS) scientists at the Arkansas Children's Nutrition Center as well as through literature searches.[231] Normal-phase HPLC, a well-studied and validated method,

was used for quantitation of PAs, from mono- to decamers, individually, and polymers greater than 10 degrees of polymerization into one peak.[232-235] Data from analytical studies identified in literature searches were only included if normal-phase or reverse-phase HPLC was conducted. Studies using the Folin-Ciocalteu and Vanillin assay methods were excluded, as they are unable to identify individual PA compounds or are not specific for PAs. Data quality was evaluated using the Nutrient Data Laboratory's Data Quality Evaluation System.

Data are presented as the sum of all forms of proanthocyanidins for a given olgomeric fraction. Monomers, dimers and trimers are reported individually, while tetramers, pentamers and hexamers are reported as 4-6mers, and heptamers, octamers, nonamers and decamers are reported as 7-10mers. Polymers (> 10 degrees of polymerization) are reported separately. All values are reported as mg/100g of fresh weight of edible portion.

Flavonoid Database for the Block 98 FFQ

NutritionQuest, the developers of the Block FFQ, assisted with the development of a flavonoid database to be used with the Block 98. In order to estimate the flavonoid intake reported by study participants on the Block FFQ, flavonoids values from the USDA Provisional Flavonoid Addendum and USDA proanthocyanidin databases had to be linked to individual FFQ food items. A variety of approaches have been reported using different FFQs.[16,17,120,218,236] In this dissertation project, the 8-digit FNDDS code was used as the predominant linking identifier as the FNDDS coding system is the current and detailed method and is used in the Provisional Flavonoid Addendum. Because the Block 98 FFQ used old 5-digit SR identifiers to identify the individual foods in the detailed

food list, the Block 98 detailed food list was recreated, with the assistance of NutritionQuest, using the 8-digit FNDDS identifiers, from the Block 2005 FFQ, which was developed to be compatible with the first version of the FNDDS. Flavonoid values from the Provisional Flavonoid Addendum were directly linked to the Block 98 FFQ using 8-digit FNDDS codes. The Proanthocyanidin Database still uses the 5-digit SR code, therefore the FNDDS-SR links file was used to link each food's 5-digit SR code to its corresponding 8-digit FNDDS code. In order to account for the contribution of a food's proanthocyanidin content to a mixed dish, the FNDDS 4.1 recipe file was used to assign appropriate proportional contribution. Proportional assignment of the other six flavonoid subclasses was already done in the creation of the Provisional Flavonoid Addendum. Figure 3.1 is a simplified schematic representation of the data linkage process. In this manner, each FFQ food item was assigned the appropriate flavonoid content in mg of flavonoid/100 gm of food.

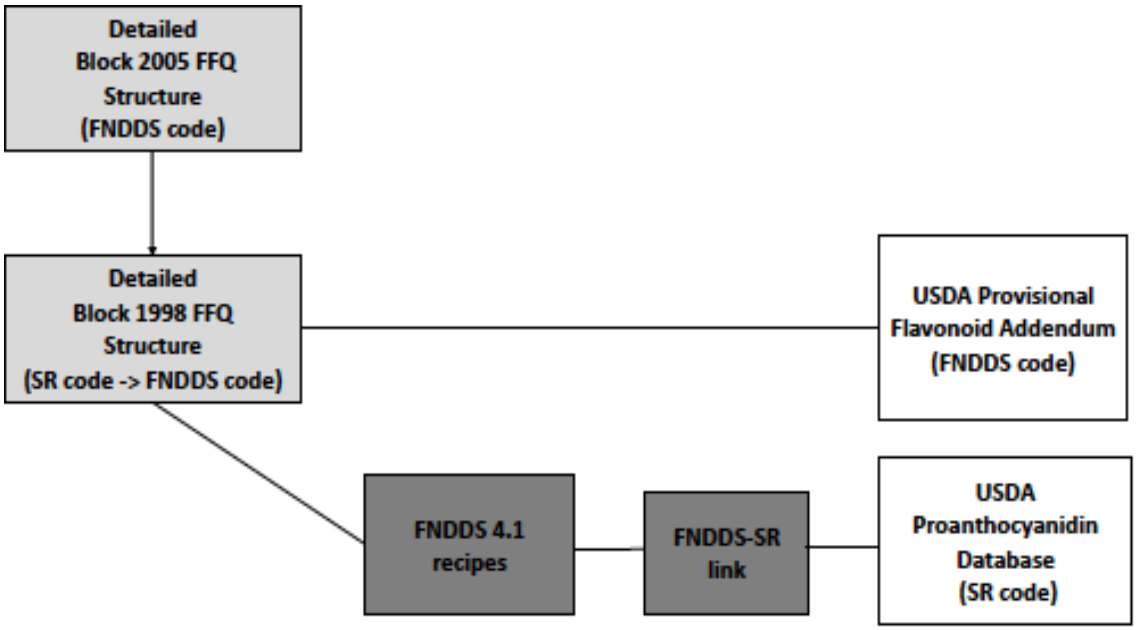


Figure 3.1 Schematic representation of data linkage between USDA flavonoid databases, USDA files and the Block 98 and Block 2005 FFQs.

The formula to assign flavonoid intake for each study participants is shown below in **figure 3.2.**

Intake of *j*th flavonoid class by i^{th} participant

$$FL\_intake_{ij} = \Sigma_k\ FL\_cont_{jk} \times food\_cons_{ik}$$

Figure 3.2 Formula for the intake of the *j*th flavonoid class by the *i*th participant.

In this formula, FL_intake_{ij} is the intake, in mg/day, of flavonoid class (*j*) by the participant (*i*), FL_cont_{kj} is the flavonoid class (*j*) amount (mg) in 100 gm of food (*k*), and $food\_cons_{jk}$ is the amount of that food (*k*) participant (*j*) reported to have consumed (mg).

An important difference between the Provisional Flavonoid Addendum and the Proanthocyanidin Database is the method by which they were constructed. While the Provisional Flavonoid Addendum was recently updated and utilizes analytical and calculated values, the Proanthocyanidin Database was released in 2004 and contains only analytically obtained values. The Proanthocyanidin Database is therefore likely to be less comprehensive. Several foods with high, expected flavonoid contents, such as apples, grapes and wine, failed to match perfectly according to their respective USDA 5-digit food code. For example, the Proanthocyanidin Database does not contain flavonoid values for SR code 09003 "Apples, raw, with skin." However, the Proanthocyanidin Database does contain analytical flavonoid values for several apple species raw, with peel, which were averaged to produce a calculated flavonoid profile that could be used. **Table 3.2** shows the foods with an imperfect match that had suitable replacement values.

Table 3.2 Proanthocyanidin Database foods with imperfect matches and suitable replacements

Food without perfect match	NDB No	Replacement Food	NDB No
Apples, raw, with skin	9003	Apples, Fuji, with peel, raw Apples, Gala, with peel, raw Apples, Golden Delicious, with peel, raw Apples, Granny Smith, with peel, raw Apples, Red Delicious, with peel, raw	97066 97067 97069 97070 97072
Grapes, red or green (European type, such as Thompson seedless), raw	9132	Grapes, green, raw Grapes, red, raw	97073 97074
Pears, raw		Pears, green cultivars, with peel, raw Pears, red anjou, with peel, raw	97075 97076
Alcoholic beverage, wine, table, all	14084	Alcoholic beverage, wine, table, red	14096 14106

		Alcoholic beverage, wine, table, white	
Peanut butter, smooth style, without salt	16398	Peanut butter, smooth style, with salt	16098

Combined food items and mixed dishes

For single food items on the FFQ, like bananas, flavonoid values were assigned directly from the USDA databases. In some cases, the Block FFQ queries items with more than one food, such as "oranges or tangerines" and "apples or pears." In these instances, a weighted average of flavonoid content was calculated using NHANES-derived population-based weights provided by NutritionQuest. For example, assuming per capita consumption of apples and pears is roughly four times as many apples as pears, the population weights were assigned as 80% and 20% for apples and pears, respectively. Flavonoid content was then assigned for that FFQ item as 80% from apples and 20% from pears. In the case of mixed dishes, such as pizza or soup, the FFQ item is assigned flavonoid content proportional to the flavonoid content of each ingredient in the USDA FNDDS, 4.1 standard recipe. Mixed dishes were disaggregated using USDA standard recipes provided in the FNDDS, 4.1 to obtain proanthocyanidin content of foods.

Data source: The REGARDS Study

The data source for the dissertation project is the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. The REGARDS study is an ongoing prospective, population-based cohort aimed at studying the causes of racial and geographic differences in stroke mortality.[237] In total 30,239 white and non-Hispanic

black participants, aged 45 years or older, were enrolled between 2003 and 2007. Community dwelling adults living within the continental United States were recruited by a combination of mail and telephone contacts using commercially available lists. Participants were oversampled from the Stroke Belt (North Carolina, South Carolina, Georgia, Tennessee, Mississippi, Louisiana, Alabama and Arkansas) such that this geographic region was represented by 56% of the study cohort. Self-reported race and sex were balanced by study design, with 42% black and 55% female participants. Participants were excluded if they (1) reported a race other than black or white, (2) reported Hispanic ethnicity, (3) were undergoing active treatment for cancer, (4) reported medical conditions that would prevent long-term participation, (5) were residents in or on a waiting list for nursing home placement, (6) experienced difficulty with hearing, articulation, comprehension of the English language or general confusion that interfered with assessment, or (7) were unable to communicate in English.

Following verbal consent, baseline data were collected by computer-assisted telephone interview (CATI) to obtain information regarding health status and medical history. Roughly one month after the CATI interview, in-home assessment were conducted by trained healthcare professionals using standardized, quality-controlled protocols to assess biometrics, obtain fasting blood and urine samples, electrocardiograms, and medication inventory by pill bottle review. Written consent was also obtained at this time and a Block 98 food frequency questionnaire (FFQ) was left behind for participants to complete. Blood samples were sent to the Central Laboratory at the University of Vermont for processing and freezing. ECG tracings were sent to a core laboratory at Wake Forest University School of Medicine for visual inspection for

technical quality. Trained physician electrocardiographers coded ECGs using the Standard Minnesota ECG classification and a second physician electrocardiographer reread all abnormalities.[28,238-241] Telephone follow-up assessments of living participants or their proxies were conducted every six months and medical records were retrieved for review of reported hospitalizations. The Questionnaire for Verifying Stroke-Free Status, which has been validated against clinical diagnosis, was used to interview participants about stroke symptoms at baseline and intercurrent events at follow-up.[242]

Dietary intake was assessed using a self-administered Block 98 Food Frequency Questionnaire (FFQ), which was left with participants with instructions for completion at the time of the in-home visit. The Block 98 FFQ is an 8-page form with more than 150 multiple-choice questions based on 107 food items, which was used to assess total energy intake, nutrient intake and grams of food intake. Participant return rate for the Block FFQ was 70%.

Study population:

Participants to be included in the analysis for **Aim 1** will be those in the REGARDS cohort who did not have a history stroke at baseline. Participants with missing demographic data, with implausible calorie intakes or who did not complete at least 85% of the FFQ will be excluded.

Participants to be included in the analysis for **Aim 2** will be those in the REGARDS cohort who did not have a history of coronary heart disease (CHD). Participants with missing demographic data, with implausible calorie intakes or who did not complete at least 85% of the FFQ will be excluded.

Participants to be included in the analysis for **Aim 3** will be those in the REGARDS cohort who did not have impaired cognitive status or a history of stroke at baseline. To be eligible, participants must have had at least two cognitive screening assessments. Participants with missing demographic data, implausible calorie intakes or those who did not complete at least 85% of the FFQ will be excluded.

Dependent Variables

The primary outcome for **Aim 1** is incident ischemic stroke. Stroke events are adjudicated using medical record review and published guidelines, a team of experts identified and adjudicated stroke events.[174] Stroke events were reported by participants or by their proxies at 6-month interval follow up interviews. A team of stroke experts adjudicated stroke events using data collected from participant medical records. A stroke was defined as focal neurological symptoms lasting > 24 hours[243] or nonfocal symptoms with positive imaging for stroke. If medical records were not available, a combination of proxy interview, death certificate and National Death Index data were used to adjudicate the stroke event.

The primary outcome for **Aim 2** is acute coronary heart disease (CHD) events. In REGARDS, CHD events are adjudicated using medical record review and published guidelines. A team of experts identified and adjudicated definite and probable myocardial infarctions.[28,238,240,241] For fatal CHD events, the participant medical history, hospital records, interviews with proxies and death certificates or National Death Index data were reviewed to determine the cause of death and whether MI was definite or probable. Two adjudicators reviewed cases with a report K>0.80 for the presence or absence of definite or probable MI.[28]

The primary outcome for **Aim 3** is global cognitive function. The REGARDS neuropsychological battery assesses three domains of cognitive function, measured on a continuous scale. These neuropsychological tests, from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) test battery, were introduced to the REGARDS study in 2006, three years after enrollment of participants began.[244] The three tests include the Word List Learning (WLL) task, Word List Delayed Recall (WLD) task[245], and the Semantic Animal Fluency Task (AFT).[246] Though follow-up was originally on an 18-month schedule, this was changed to a 2-year follow-up schedule in February 2008.

Table 3.3 REGARDS CERAD-battery tests

Cognitive Domain	Psychometric Test	Scoring	Test schedule
Learning	Word List Learning (WLL)	Sum of 3 learning trials (0-30)	Biennial starting with month 18 (months 18, 42, 66, 90, 114)
Memory	Word List Delayed Recall (WLD)	Number correct (0-10)	
Executive	Animal Fluency Task (AF)	Number correct in 60 seconds; number of intrusions	

The WLL and WLD represent the cognitive domains of learning and memory, respectively, and can be used separately. The Word List Learning task entails three learning trials of a list of 10 semantically unrelated words, presented in a fixed order, varying across the three trials. Recall follows each trial and the score for the WLL is the sum of correctly recalled words, excluding repetitions and intrusions (inclusion of words not on the list). Instructions for list learning are administered via recording so that all participants are exposed to the same narration. The Word List Delayed Recall, is administered after a 5-minute delay filled with non-cognitive interview questions.[244]

Participants are asked to recall freely as many words from the WLL as possible. Scores range between 0-10 for the number of words correctly recalled. The Animal Fluency Task (AF) is a measure of executive function. Participants are asked to name as many animals as possible in 60 seconds. The score is the number correct in 60 seconds minus the number of intrusions (not an item among the material that was presented to be remembered, in this case, not an animal). College-educated scorers, following scoring protocols using computer-assisted programs, score neuropsychological assessments. Interscorer agreement between college-educated scorers and expert scorers is excellent (kappa > 0.95).[244] A global cognitive score was calculated by first obtaining a z-score for each of three cognitive tests based on the study population. Available z-scores at each cognitive assessment time point were averaged to obtain an average global cognitive score.

Data analysis plans

Dietary flavonoid intake will be divided into quintiles, with the lowest quintile (lowest dietary flavonoid intake) as the reference. If there is a moderate to strong correlation found between flavonoid intake and energy intake, then energy-adjusted residuals[247] will be used in place of absolute flavonoid intakes. In Aim 1, the association between flavonoid intake and the primary outcome of interest, incident ischemic stroke, will be examined using Cox proportional hazard models, adjusting sequentially for predetermined covariates in conceptually related groups. First crude associations will be estimated (Model I) followed by: adjustment for age and energy intake (Model II), demographic factors (Model III) including sex, race, and region of residence; additional adjustment for environmental factors (Model IV) including education, income, physical

activity, and smoking status. Assessment of confounding by diet will include factors such as percentage of calories from sweetened foods and beverages, whole grains, fatty acids, fiber, folate, vitamin C, and β-carotene. Because dietary variables can be strongly intercorrelated, they will be added to the model individually and retained if they were independently associated with incident stroke or changed the HR in the top quintile of exposures greater than 10%. Cardiovascular risk factors, including BMI, blood pressure, diabetes mellitus and hyperlipidemia as well as history of CHD will be included in the model in exploratory analyses, as these factors are likely to be intermediates on the causal pathway between flavonoid intake and incident ischemic stroke. (see **figure 3.3**)

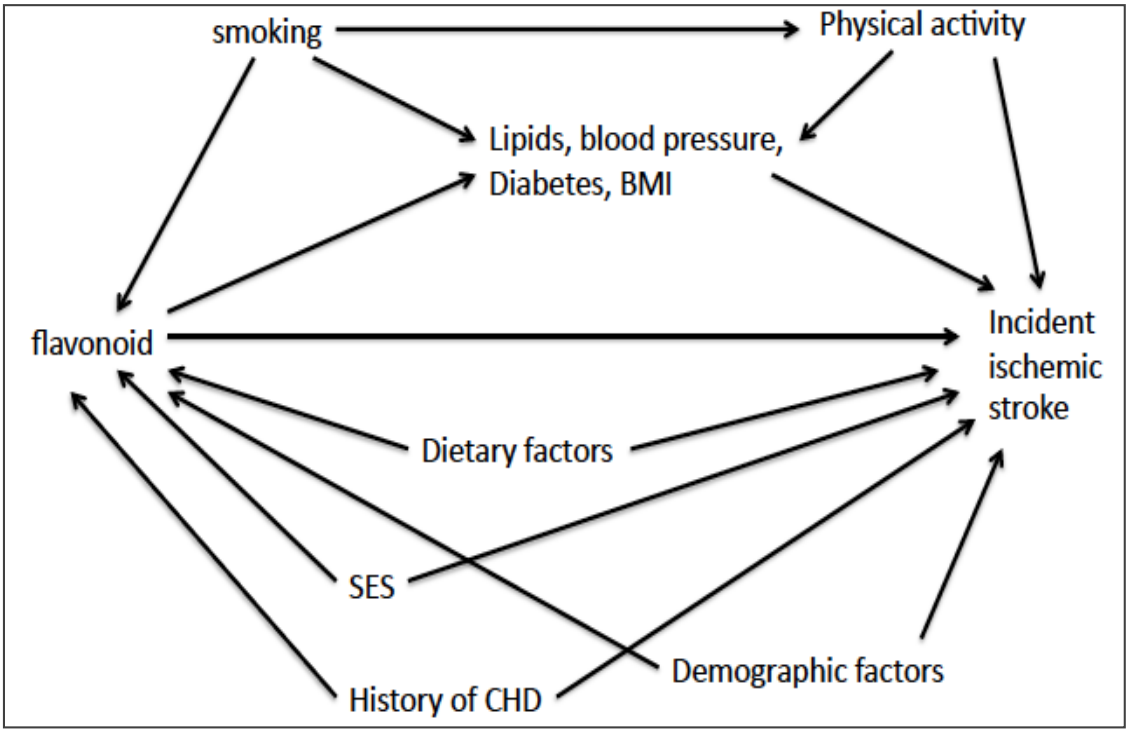


Figure 3.3 Simplified directed acyclic graph for Aim 1 indicating associations between flavonoid intake and incident ischemic stroke.

From the Cox proportional hazard analyses, the hazard ratio (HR) with corresponding 95% confidence intervals (CI) for each flavonoid quintile compared to the

reference will be calculated. Collinearity among covariates will be tested. A condition index ≥ 30 coupled with a variance decomposition proportion of 0.5 or greater will be considered evidence of collinearity. The proportional hazards assumption will be assessed by comparing –ln(ln) curves. For interaction analyses, -2 ln likelihood tests for models with and without interaction terms will be used. Tests for linear trend will be conducted by using the median values of each flavonoid intake quintile, analyzing as a continuous variable.

In Aim 2, we will examine the association between flavonoid intake and the primary outcome of interest, incident CHD, using Cox proportional hazard models, adjusting sequentially for predetermined covariates in conceptually related groups, similar to Aim 1. A simplified directed acyclic graph for Aim 2 indicating associations between flavonoid intake and incident CHD is in **figure 3.4**.

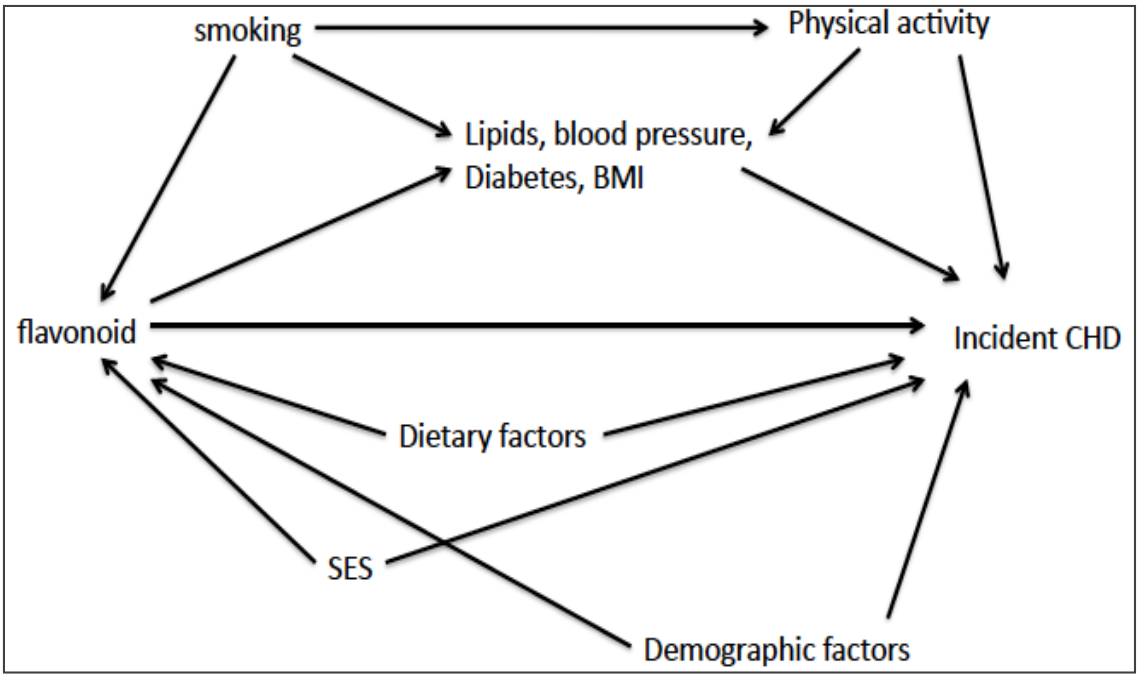


Figure 3.4 Simplified directed acyclic graph for Aim 2 indicating associations between flavonoid intake and incident CHD.

Assessment for collinearity and stratified analyses will be conducted as for Aim 1. Additionally, in Aim 2 we consider a sensitivity analysis in which participants are excluded based on Goldberg cut-off criteria.[248,249] This sensitivity analysis seeks to investigate the effect of energy misreporting by excluding those participants whose reported energy intake is represents an underestimation of energy needs based on their predicted basal metabolic rate. The basal metabolic rate is estimated using the Schofield equations for height, weight, and age.[250]

For Aim 3, the association between flavonoid intake and global cognitive function will be assessed in a repeated measures analysis. Linear mixed models (PROC MIXED in SAS) will be used to evaluate the association between flavonoid intake and changes in the composite cognitive score over time, allowing for correlation within participant cognitive measures over time and allowing for varying numbers of cognitive assessments. All models included a term for flavonoid intake, time and the interaction between flavonoid intake and time. Time was modeled as a nominal variable (reflecting the cognitive assessment occasion), as opposed to a continuous variable, to avoid assumptions about shape of cognitive function trends over time. The β-coefficient for interaction terms with time represents the estimated effect of the variables on the rate of change in cognitive score. Sequential adjustment will proceed as in Aims 1 and 2, with additional adjustment for depressive symptoms. Prevalent stroke is considered a mediator of the association between flavonoid intake and cognitive function. The simplified directed acyclic graph is shown in **figure 3.5**.

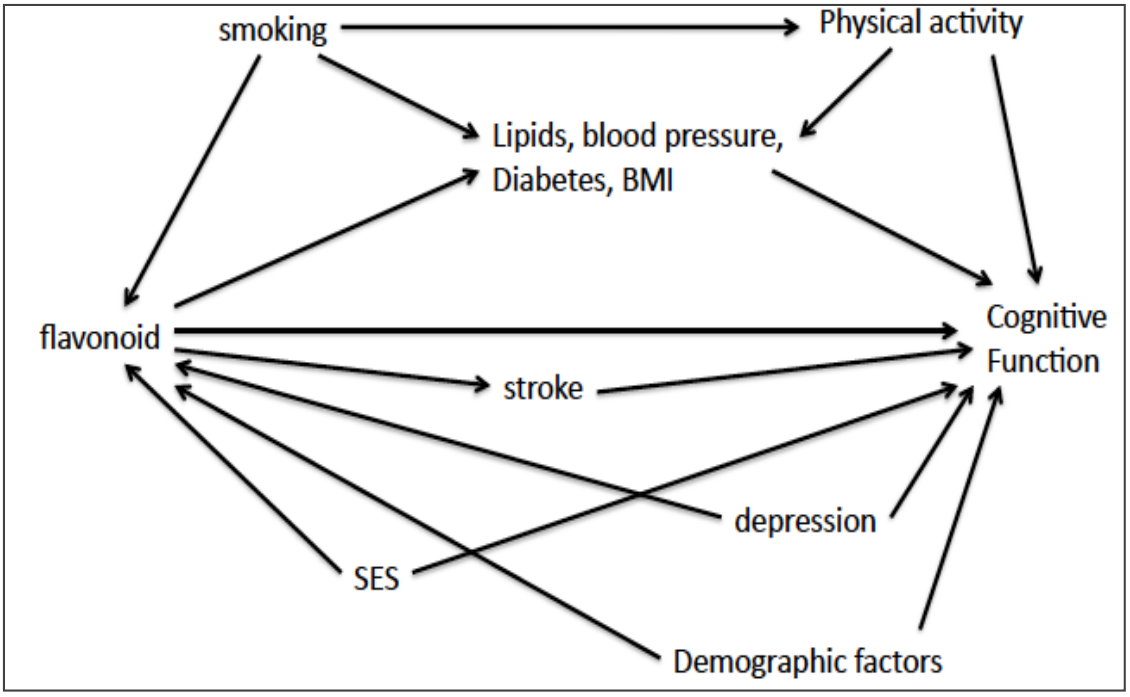


Figure 3.5. Simplified directed acyclic graph for Aim 3 indicating associations between flavonoid intake and cognitive function.

To evaluate potential effect modification by age (dichotomized at 65 years), sex, self-reported race, and region of residence we will examine 2- and 3-factor interaction terms among covariates (age, sex, race and region of residence), flavonoid intake and time. Lower-order terms were retained as needed to maintain hierarchically well-formulated models. Several covariance structures will be compared, including compound symmetry, first-order autoregressive, Toeplitz and unstructured. Covariance structure will be selected based on biologic plausibility and the lowest Akaike information criterion.

To complement findings for flavonoids and outcomes of interest, we will conduct food-based analyses to examine the association between selected FFQ food items and our outcomes of interest using multivariable models similar to those used for flavonoid intake. We included foods if they comprised at least 1% of reported total flavonoid intake

or if the food was previously determined, in scientific literature, to be associated with cognitive health. These foods included tea (52% of total flavonoid intake), apples or pears (6%), cakes (5%), citrus fruits/juices (5%), canned fruit (3%), wine (3%), 100% fruit juice excluding orange (3%), peanuts/nuts (2%), legumes (2%), berries (1%), cocoa (<1%) and tofu (<1%).

A two-sided p-value of less than 0.05 will be considered as evidence of statistical significance. All statistical analyses will be performed with the SAS version 9.3 or 9.4 (SAS Institute, Cary, NC) statistical software package.

CHAPTER 4. REPORTED FLAVANONE INTAKE IS ASSOCIATED WITH RISK OF INCIDENT ISCHEMIC STROKE IN THE REGARDS STUDY

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ABSTRACT

Background: Flavonoids may have beneficial cerebrovascular effects in humans, but studies to date are limited by a lack of comprehensive flavonoid databases and lack of racially and geographically representative cohorts. Given racial and geographic disparities in stroke, representative cohort studies are needed.

Objectives: We evaluated the association between flavonoid intake and incident ischemic stroke in a biracial, national cohort, using updated flavonoid composition tables, and assessed differences in flavonoid intake by sex, race and geographic region of residence.

Methods: We evaluated 20,024 participants in the REasons for Geographic and Racial Differences in Stroke (REGARDS) Study, a biracial prospective study. Participants with stroke history or missing dietary data were excluded. Flavonoid intake was estimated using a Block98 food frequency questionnaire and the United States Department of Agriculture's Provisional Flavonoid Addendum and Proanthocyanidin Database. Associations between quintiles of flavonoid intake and ischemic stroke were evaluated using Cox proportional hazards models, adjusting for confounders.

Results: During 6.5 years of follow-up, 524 acute ischemic strokes occurred. Flavanone intake was lower in the Southeastern U.S. but higher in blacks compared to whites. After multivariable adjustment, high versus low flavanone intake was associated with a statistically significant reduced risk of incident ischemic stroke (HR: 0.73; 95% CI: 0.55, 0.95; *P*-trend=0.03). Greater consumption of citrus fruits and juices, the primary dietary source of flavanones, was inversely associated with incident ischemic (HR: 0.70; 95%

CI: 0.54, 0.91, *P*-trend=0.02). There was no statistical interaction for any flavonoid measure.

Conclusions: Greater consumption of flavonones, but not total or other flavonoid subclasses, was inversely associated with incident stroke. Associations did not differ by sex, race or geographic region for the association, however regional differences in flavanone intake may contribute to regional disparities in ischemic stroke. Higher flavanone intake in blacks suggests that flavanone intake is not implicated in racial stroke disparities.

Keywords: ischemic stroke, flavonoid, plant-based diet, polyphenol, epidemiology

INTRODUCTION

Flavonoids are bioactive, polyphenolic compounds widely distributed in plants. Flavonoids are believed to be implicated in the beneficial properties of plant-based diets towards cardiovascular health, including stroke [13]. Proposed cardioprotective mechanisms for flavonoids include antioxidant and anti-inflammatory action, modulation of lipid metabolism and platelet function and attenuation of hypertension [14]. Consistent with proposed biologic mechanisms, results from epidemiologic studies suggest a protective effect for flavonoids against cardiovascular disease (CVD) mortality [13,16,17], incident coronary heart disease (CHD) [18,19], and incident stroke [20] as well as other indicators of cardiovascular risk, such as arterial stiffness, incident hypertension, and type 2 diabetes [21-24]. While dietary flavonoid research has progressed over the past decade, existing literature is limited by incomplete dietary flavonoid information and lack of geographic and racial/ethnic diversity of studied populations, especially in the context of incident stroke.

A lack of comprehensive dietary flavonoid composition tables has especially limited previous research. The use of diet composition tables with missing or incomplete information of flavonoid concentration in some foods or mixed dishes may have led to underestimation of flavonoid intake. Conversely, imputation of values in cooked dishes from raw foods, without adjusting for processing losses, may have led to overestimation. To address these limitations, the U.S. Department of Agriculture (USDA) released the Provisional Flavonoid Addendum. The Provisional Flavonoid Addendum provides flavonoid values for an expanded number (over 7,000) of foods and beverages, and uses more precise estimation methods for flavonoid content in mixed and cooked dishes [25].

A second limitation of existing literature is a general lack of racial and regional diversity in the previous studies. Black Americans and residents of the Southeastern United States, a region also known as the Stroke Belt, bear a higher burden of stroke risk than whites and those living elsewhere in the U.S. [26,27]. Since traditional risk factors account for only 50% of the racial disparity in stroke, a variety of nontraditional risk factors, including dietary habits, may contribute to these disparities [36,176]. A Southern-style diet, characterized by high intakes of meats and fried foods, is associated with greater stroke risk, and those with high adherence to a Southern-style diet are more often black or residents of the Southeastern U.S [7]. In contrast, high adherence to a plant-based diet is associated with lower stroke risk [7,9,251,252]. Differences in dietary flavonoid intake may help to explain racial and regional differences in stroke, but only a few previous studies compared dietary flavonoid consumption by race [34,35] and none by region in the United States.

The Reasons for Geographic and Racial Differences in Stroke (REGARDS) study is a national biracial prospective cohort study designed to investigate racial and regional disparities in stroke. Using the USDA's expanded flavonoid database, the Provisional Flavonoid Addendum, and data from REGARDS, we evaluated the association between total flavonoid and flavonoid subclass intakes and incident acute ischemic stroke. Given the REGARDS study's unique design, we also assessed differences in flavonoid consumption by race and region.

METHODS

Participants

The REasons for Geographic and Racial Difference in Stroke (REGARDS) study is a prospective cohort study designed to study racial and geographic differences in stroke. Between 2003 and 2007, 30,239 community-dwelling adults, ≥45 years old and living in the continental United States, were recruited into the study. The study design has been described in detail previously [237]. Participants were oversampled from the Southeastern region of the U.S., referred to as the "Stroke Belt", including Alabama, Arkansas, Georgia, Louisiana, Mississippi, Tennessee, North Carolina and South Carolina. Within the Stroke Belt, the coastal plains regions of Georgia, North Carolina and South Carolina, often referred to as the Stroke Buckle, experience an even higher rate of stroke mortality than the rest of the Stroke Belt. Self-reported race and sex were balanced by design resulting in a cohort with 56% stroke-belt residents and 44% residents of the remaining contiguous lower 48 states, 42% black and 55% female participants [174]. Information about age, residential region, education, income, smoking status, physical activity, baseline health status and medical history, including stroke risk factors, such as hypertension, diabetes and obesity status, was collected by computer-assisted telephone interview. Trained healthcare professionals conducted in-home visits to perform standardized measures of risk factors and collect blood and urine samples. The Institutional Review Boards of all participating universities approved this study. Written informed consent was obtained from all participants.

Dietary Assessment

A self-administered Block98 Food Frequency Questionnaire (FFQ) was left with study participants at the in-home visit. The Block98 FFQ is a 107-item questionnaire, developed by NutritionQuest (Berkeley, CA) and has been validated in populations

similar to REGARDS [213,214]. Though not specifically designed to assess flavonoid intake, this FFQ inquires about intakes of flavonoid-rich foods, including fruits, vegetables, tea, and wine and has been used to demonstrate a diet-disease relationship for flavonoid intake and breast cancer outcomes [219].

Flavonoid Intake

Flavonoid intakes of interest were total flavonoid intake, anthocyanidins, flavan-3-ols, flavanones, flavone, flavonols, isoflavones, and proanthocyanidins. Flavonoid content was obtained from the USDA Database for the Proanthocyanidin Content of Selected Foods and the USDA's Provisional Flavonoid Addendum to the USDA Food and Nutrient Database for Dietary Studies (FNDDS), 4.1 [25,46]. **Table S.4.1** summarizes the flavonoid compounds included in each database. The newly-released Provisional Flavonoid Addendum, contains data for 29 flavonoids in six flavonoid subclasses for 7,174 foods/beverages in the FNDDS, 4.1 and accounts for processing and cooking effects better than previous USDA flavonoid databases [25]. In contrast, the USDA Database for the Proanthocyanidin Content of Selected Foods, released in 2004, has not been updated [46]. The Provisional Flavonoid Addendum and Proanthocyanidin Database are largely complementary; though contain overlapping information about flavan-3-ols, identified as monomers in the Proanthocyanidin Database. Given methodological differences between databases, when a food was included in both databases, and if a flavan-3-ol value (Addendum), and monomer value (Proanthocyanidin Database) were both available, only the flavan-3-ol value from the Provisional Flavonoid Addendum was used. Thearubigin intake, which is exclusively from black tea, was excluded from flavan-3-ol and total flavonoid estimates in the main analysis, though included in sensitivity

analyses, because there is no consensus on analytic estimation methods for thearubigins [253]. The association between daily tea consumption (defined as mean intake of least one cup of tea daily), which can be considered a marker of thearubigin consumption, was examined separately.

Food items on the Block98 FFQ were linked to the Provisional Flavonoid Addendum and Proanthocyanidin Database using 8-digit FNDDS and 5-digit Standard Reference food codes, respectively. The sum of flavonoid compounds was calculated for each flavonoid subclass. For combined items on the FFQ, such as "apples or pears," a weighted average of flavonoids was calculated using population-based weighted intakes, consistent with the Block 98 FFQ. Estimated daily flavonoid intake per participant equaled the reported amount (grams) of food consumed multiplied by the flavonoid content of the corresponding food (mg flavonoid/100 grams of food) and summed across foods.

Stroke Ascertainment

Telephone surveillance, every six months, was used to assess vital status and gather information about reasons for hospitalization, including stroke, transient ischemic attack and stroke symptoms. Medical records were retrieved for suspected strokes, followed by central physician adjudication of stroke events. Stroke is defined: (1) focal neurologic deficit lasting > 24 hours or (2) nonfocal neurological symptoms with brain imaging consistent with stroke [174]. The National Death Index was queried annually to identify stroke deaths that might not have been hospitalized. Incident strokes occurring up to September 31, 2013, were included.

Statistical Analyses

All analyses were conducted in SAS version 9.3 (SAS Institute). We categorized flavonoid intakes into quintiles to avoid assumptions about the dose-response relationship and because quintiles offered good separation of highest and lowest intake with ample power. We summarized baseline characteristics by quintiles of flavonoid intake. Given methodological differences in the creation of the Provisional Flavonoid Addendum and the Proanthocyanidin Database, two total flavonoid intake variables were examined, one including only flavonoid values found in the Provisional Flavonoid Addendum (total flavonoid I) and another adding proanthocyanidin intake (total flavonoid II). Correlations between nutrients were explored using Pearson and Spearman correlations.

Cox proportional hazards models were used to examine the association between quintiles of flavonoid intake and risk of incident AIS. Participants were censored at date of stroke, loss to follow-up or last stroke adjudication, whichever occurred first. After verifying the proportional hazards assumption, models were built sequentially. The base model with an unadjusted model followed by model 1, which included continuous age, continuous energy intake and sex. In model 2 demographic variables were added including self-reported race (non-Hispanic black or white), region of residence (Stroke Belt, Stroke Buckle or non- Stroke Belt) and an age-race interaction term. The age-race interaction term was included in the model to account for differences in stroke incidence between blacks and whites at younger ages [174]. Model 3 included yearly income (categorized as <$20,000, $20,000-34,999, $35,000-74,999, ≥$75,000, or refused) and education (categorized as < HS, high school, some college, college and above). Model 4 included health behaviors (physical activity and smoking status) and history of coronary artery

disease at baseline. Physical activity was categorized as none, 1-3 times/week, or ≥4 times/week and smoking status was categorized as never, former or current. Statistical interaction by demographic variables self-reported race (non-Hispanic black, white), sex (men, women) and region (Stroke Belt and Stroke Buckle combined versus non-Stroke Belt as well as Stroke Belt, Stroke Buckle, non-Belt, separately) was tested using likelihood ratio tests. Trend tests were conducted by assigning each quintile its median value and modeling the exposure as a continuous variable. In additional analyses we added dietary variables to the models, including whole grains, fatty acids, fiber, folate, vitamin C, and β-carotene. Because dietary variables can be strongly intercorrelated, they were added to the model individually and retained if they were independently associated with incident stroke or changed the HR in the top quintile of exposures greater than 10%. Several sensitivity analyses were conducted. Analyses involving total flavonoids and flavan-3-ols were repeated including thearubigins to facilitate comparison with other flavonoid studies that include them. We also considered alternate categorizations of flavonoid intake, including quartiles and deciles, to verify findings using quintiles. Sensitivity analysis using multiply-imputed missing covariates was conducted to assess possible bias due to 1) reporting of income as "refused" (n=2,291) and 2) exclusion of participants with missing information on three covariates; smoking (n=78), education (n=291), physical activity (n=9) or some combination (n=11). Using the Monte Carlo Markov chain method for missing at random assumptions and the available covariate data, we created 5 imputed datasets with PROC MI in SAS. Parameter estimates from each imputed dataset were pooled using PROC MIANALYZE. $P < 0.05$ was considered statistically significant in all analyses.

Results

Complete dietary information with plausible energy intake (500-5000 kcal/day) was available for 21,636 participants. After excluding participants with a history of stroke (n=1223) or missing information on key covariates (n=389), 20,024 participants (56% women), remained for this analysis. The study flow diagram is shown in **Figure 4.1**. During a mean follow-up of 6.5 years, 524 incident AIS events were observed, 250 in women, and 274 in men. There were no differences by important characteristics, except that FFQ returners were more often black (61.3% versus 38.7%), less educated (20% versus 10% with < high school) and had lower income (24.0% versus 15.8% < $20,000). On average, white participants consumed a greater number of servings of vegetables than black participants (3.2 ± 2.2 servings/day vs 2.6 ± 2.1 servings/day, P<0.0001) but there was no difference in servings of fruits consumed. Those living outside of the stroke belt, on average, consumed more servings of fruits than those in the stroke belt or the stroke buckle (1.5 ± 1.1 servings/day, 1.3 ± 1.0 servings/day, 1.3 ±1.0 servings/day, respectively; P<0.0001) but there was no difference in the number of servings of vegetables consumed.

The top food sources of flavonoids, by subclass, are shown in **Table S.4.2**. Individuals with higher total flavonoid intake were more likely to be white, more educated, less sedentary, have higher household income, and never to have smoked **Table 4.1**. The distribution of baseline characteristics did not differ using total flavonoid II. The distribution of sex, race and region of residence are shown in **Table 4.2**. Total flavonoid and flavonoid subclass intakes were higher among white participants as compared to their black counterparts, except for flavanone. Anthocyanidin, flavanone, and isoflavone

intakes tended to be lower among those living in the Stroke Belt and Stroke Buckle than living elsewhere. The Spearman rank correlation between total flavonoid intake and energy intake was low ($r=0.24$, $p<0.001$); therefore energy-adjusted residuals were not used, but total energy intake was included in final models.

Associations between flavonoid intake and incident AIS are shown in **Table 4.3**. There was a statistically significant inverse association between flavanone intake and risk of AIS emerging at the second quintile in multivariable-adjusted models with a statistically significant linear trend. In unadjusted models, a significant inverse association with isoflavone intake emerged at the second quintile of intake, though this was not significant in multivariable-adjusted models. There were no consistent associations between total flavonoid, anthocyanidin, flavan-3-ol, flavone, flavonols or proanthocyanidins and stroke. When thearubigin intake was included in calculations of total flavonoid and flavan-3-ol intake, results were materially unchanged (data not shown). There was no statistically significant association between daily tea consumption and incident ischemic stroke (HR: 1.09; 95% CI: 0.89, 1.34).

Because citrus fruits/juices are the main dietary source of flavanones, vitamin C and potassium were added to the multivariable models to account for their potential beneficial effect. In the total cohort, comparing extreme quintiles of flavanone intake, the hazard ratio was unchanged by adding vitamin C (HR: 0.76; 95% CI: 0.53, 1.11) or potassium (HR: 0.74; 95% CI: 0.55, 0.98). When considering quintiles of citrus fruit and juice intake as the exposure, instead of flavanone intake, the highest quintile of citrus fruits and juices intake was inversely associated with incident AIS in the total cohort, in multivariable adjusted models (HR_{Q2vQ1}: 0.89; 95% CI: 0.68, 1.15; HR_{Q3vQ1}: 0.74; 95%

CI: 0.56, 0.97; HR_{Q4vQ1}: 0.82; 95% CI: 0.63, 1.07; HR_{Q5vQ1}: 0.70; 95% CI: 0.54, 0.91; *P*-trend=0.02). Median intakes of citrus fruits and juices for each quintile, in ascending order, were 5.1 gm/day, 21.0 gm/day, 60.8 gm/day, 142.3 gm/day, 266.9 gm/day. In post-hoc analysis, effect estimates for comparisons of extreme quantiles were similar to results from quintiles (quartiles HR_{4vs1}: 0.78; 95% CI: 0.61, 0.99 and deciles HR_{10vs1}: 0.76; 95% CI: 0.51, 1.13). Considering 10 gm increments of flavanone intake there was a 5% decrease in relative risk of incident ischemic stroke for each additional 10 gm of flavanone consumed (HR: 0.95; 95% CI: 0.92, 0.98). When all dietary variables evaluated for potential confounding were included in the model, the effect estimate for flavanone intake changed minimally, though the confidence interval widened (HR: 0.78; 95% CI: 0.53, 1.14, **Table S.4.4**). There was no statistically significant interaction by sex, self-reported race or region of residence in flavonoid analyses (all *P*-interaction > 0.40) Stratified analyses are in **Table 4.4, Table 4.5 and Table S.4.3**. Tests for statistical interaction by region of residence did not differ when the Stroke Belt and Stroke Buckle were considered separately or combined and are presented in Table 4 as combined. Results from sensitivity analyses using multiply-imputed missing covariates were materially unchanged from the complete case analysis.

Discussion

This is the first study, in a biracial U.S. population, to evaluate the association of total flavonoid and seven flavonoid subclass intakes with incident AIS, using an extensive contemporary database, the USDA's Provisional Flavonoid Addendum [25]. This expanded flavonoid database provides detailed information on flavonoid content, across six subclasses, for a large number of foods and beverages, including multi-ingredient and processed foods, enabling a more comprehensive evaluation of flavonoid intake and

incident AIS than ever done before. Our study suggests that specific subclasses of flavonoids are more relevant to incident AIS than total flavonoid intake. Higher flavanone intake was associated with a 37% decreased relative risk of incident AIS after adjustment for potential confounders. These findings were robust to different categorizations of flavanone intake. There was a similar inverse association for higher intakes of citrus fruits/juices, the primary dietary source of flavanones. These findings are consistent with the Nurses' Health Study, which found a 19% reduction in risk of incident AIS associated with high flavanone intake, using a previous version of the USDA flavonoid databases [20]. An inverse, though non-significant, association between flavanone intake and stroke mortality has also been described [16,17]. Furthermore, consumption of fruits, especially citrus fruits, an important source of flavanones, has been associated with fewer AIS events [251,254-256].

Flavanones are associated with improved microvascular reactivity, lower blood pressure, improved FMD and improved lipid profiles [257,258]. Naringenin and hesperitin, both flavanone compounds, are potential candidates for neuroprotection, as they cross the blood-brain barrier in animal models, inhibiting inflammatory signaling and cytokine production [259,260]. These salutary vascular and anti-inflammatory effects, paired with their ability to cross the blood-brain barrier, make flavanones biologically plausible candidates for neuroprotection.

After multivariable adjustment we found no statistically significant association between total flavonoid or flavonoid subclass intakes other than for flavanones. Greater intakes of anthocyanidins, flavan-3-ols, proanthocyanidins and total flavonoids have been inversely associated with stroke events in men and women (5-19% risk reduction) though failed to

reach statistical significance [16,20]. However, in a metaanalysis, all of these flavonoid subclasses were found to have a 10-13% relative reduction of risk of combined fatal and nonfatal cardiovascular disease [261]. Differences between the effect estimates obtained from our study and metaanalyses could be that this study focused on adjudicated incident AIS rather than combined fatal and nonfatal cardiovascular disease in the metaanalysis. In this study, flavones and flavonols were not associated with incident AIS. This lack of an association is consistent with the preponderance of previous epidemiologic studies of incident stroke and stroke mortality [13,16,20]. However, in two previous metaanalyses, flavonols were inversely associated with incident AIS or combined fatal and nonfatal cardiovascular events [261,262]. Conflicting findings from observational studies and metaanalyses may be due to differences in statistical power and different outcome definitions.

Although there was no significant effect modification by sex, race or region of residence, black participants reported lower intakes of all flavonoid subclasses except flavonones than their white counterparts. These differences are consistent with previous studies of flavonoid subclass intakes in U.S. adults [34]. Furthermore, the lower intake of flavonoids, except for flavanones, was consistent with lower average intake of vegetables among black participants in this study. Given the inverse association between flavonone intake and incident stroke and that black participants in this study consumed more flavanones than their white counterparts, it is unlikely that differences in flavanone intake explain racial disparities in stroke. Racial disparities may be related to non-flavonoid nutrients that are deficient in diets otherwise low in fruits and vegetables. Regional disparities in stroke may be related to lower flavanone intake in residents of the Stroke Belt and Stroke

Buckle. One published study addressed modification of the association between isoflavone intake and blood pressure by race, suggesting stimulation of eNOS in the setting of relative NO deficiency, as a potential mechanism to explain a stronger beneficial association between dietary isoflavone and blood pressure in black participants compared to white participants [24]. Other flavonoid subclasses have been implicated in nitric oxide modulation [77], but no other studies in humans have been published that allow comparison of results.

Strengths of this study include the large sample and prospective design, including racially and geographically diverse participants with excellent ascertainment of stroke outcomes. Furthermore, this study utilized the most comprehensive flavonoid database available to assess dietary flavonoid intake in the U.S. population. There are limitations to this study worth mentioning. By using a comprehensive flavonoid database with a detailed FFQ, our study likely captured the most significant sources of dietary flavonoids. The Provisional Flavonoid Addendum was recently used to characterize flavonoid intake and important dietary sources of flavonoids in the U.S. population in the What We Eat in America, NHANES 2007-2008 study [263]. Important dietary sources were defined as those that contributed at least 5% of the intake of a flavonoid subclass or total flavonoids. The only important dietary source of flavonoids not specifically listed on the Block98 FFQ or included as a part of mixed dishes or aggregate FFQ items, was soy protein powder, which contributed 32% of isoflavone intake. While this is a limitation in for isoflavone analyses, the contribution of isoflavone intake to total flavonoid intake in the U.S. is very low. Four other important dietary sources of flavonoids, red/purple vegetables (8% of anthocyanidins), sweet peppers (9% of flavones), celery/squash (8% of flavones), onions

(5% of flavonols), were included in the Block98 as a part of mixed dishes or aggregate FFQ items. These four foods were all represented in the USDA recipes used to develop nutrient descriptions for the 7,174 foods and beverages in the Provisional Flavonoid Addendum. Furthermore, since onions, sweet peppers, red/purple vegetables and celery/squash are often consumed as a part of mixed dishes rather than in isolation, using standard recipes to estimate their intake may improve measurement rather than relying on respondents to correctly report the proportionate amount of a vegetable, such as onion, consumed as a part of a dish. Second, dietary assessment was conducted only at baseline; therefore changes in dietary intake of flavonoids were not assessed. Because this analysis was conducted in a dietary subsample, selection bias is a concern. However, the return rate for the FFQ in the REGARDS study was 70%, which is higher than other self-administered questionnaire return rates. Incident AIS rates were the same comparing those who did and did not return the FFQ, and they were similar on the majority of characteristics. Non-returners were more likely to be black, less educated and poorer. Because these characteristics were not found to modify the association between flavonoid intake and risk incident AIS, suggesting homogeneity for the association across strata, bias is unlikely to result from the exclusion of those participants. There was also no difference between results obtained from the complete case analysis as compared to analyses with imputed values for missing covariates. Though we considered many potential confounders, residual and unmeasured confounding is always a concern in observational studies. Finally, due to multiple comparisons, statistical significance should be interpreted cautiously and in the context of existing literature.

Conclusions

We found that a higher reported dietary intake of flavanones is associated with a 27% lower risk of incident AIS. Moreover, greater citrus intake, the predominant source of flavanones, is associated with a reduction in the risk of AIS. While there was no effect modification by race or region of residence, lower flavanone intake in the Stroke Belt and Stroke Buckle may contribute to excess stroke burden in residents of the Stroke Belt and Stroke Buckle. Lower vegetable and flavonoid consumption, except for flavanones, in black participants suggests that non-flavonoid dietary factors may be more important than flavonoids to explain the excess stroke risk in this group.

Table 4.1. Baseline characteristics for 20,024 stroke-free participants in the REasons for Geographic and Racial Differences in Stroke (REGARDS) Study 2003-2007 by Quintile of Total Flavonoid Intake (total I)[1]

	Quintile of Total Flavonoid I intake					
	Q1	Q2	Q3	Q4	Q5	
Median intake (range) mg/day	34.31 (≤ 50.86)	66.6 (50.9-83.4)	102.9 (83.4-127.1)	156.9 (127.1-208.3)	296.84 (≥ 208.33)	p^2
n	4005	4005	4005	4004	4005	
Age, y	63.6 ± 9.1	64.4 ± 9.2	65.1 ± 9.2)	65.4 ± 9.4)	64.7 ± 9.1)	0.002
Energy intake, kcal	1396 ± 571	1595 ± 648	1751 ± 689	1846 ± 699	1960 ± 789	<0.001
Female, n (%)	2288 (57.1)	2241 (56.0)	2224 (55.5)	2213 (55.3)	2287 (57.1)	0.34
White, n (%)	2394 (59.8)	2485 (62.1)	2580 (64.4)	2782 (69.5)	3124 (78.0)	<0.001
Region,[3] n (%)						<0.001
Stroke Belt	1426 (35.6)	1288 (32.2)	1323 (33.0)	1378 (34.4)	1480 (37.0)	
Stroke Buckle	765 (19.1)	831 (20.8)	805 (20.1)	901 (22.5)	1070 (26.7)	
Non-belt	1814 (45.3)	1886 (47.1)	1877 (46.9)	1664 (43.1)	1397 (36.3)	
Physical activity, n (%)						<0.001
None	1539 (38.4)	1322 (33.0)	1202 (30.0)	1133 (28.3)	1216 (30.4)	
1-3 times/wk	1441 (35.9)	1494 (37.3)	1540 (38.5)	1535 (38.3)	1467 (36.6)	
≥4 times/wk	1025 (25.6)	1189 (29.7)	1263 (31.5)	1336 (33.4)	1322 (33.0)	
Smoking status, n (%)						<0.001
Never	1608 (40.2)	1774 (43.6)	1908 (47.6)	1928 (48.2)	1948 (48.6)	
Former	1603 (40.2)	1629 (42.3)	1651 (41.2)	1660 (41.5)	1575 (39.3)	
Current	794 (19.8)	569 (14.2)	454 (11.1)	427 (10.4)	515 (12.0)	
Education, n (%)						<0.001

<HS	487 (12.2)	372 (9.3)	333 (8.3)	304 (7.6)	324 (8.1)	
HS grad	1176 (29.4)	1039 (25.9)	904 (22.6)	935 (23.4)	1016 (25.4)	
Some college	1141 (28.5)	1107 (27.6)	1077 (26.9)	1055 (26.4)	1130 (28.2)	
College grad	1201 (30.0)	1487 (37.1)	1691 (42.2)	1710 (42.7)	1535 (38.3)	
Income, n (%)						<0.001
Refused	454 (11.3)	486 (12.1)	457 (11.4)	439 (11.0)	455 (11.4)	
<$20,000	752 (18.8)	599 (15.0)	566 (14.1)	538 (13.4)	570 (14.2)	
$20,000-$34,000	1016 (25.4)	940 (23.5)	896 (22.4)	989 (24.7)	944 (23.6)	
$35,000-$74,000	1193 (29.8)	1279 (31.9)	1352 (33.8)	1270 (31.7)	1278 (31.9)	
≥$75,000	590 (14.7)	701 (17.5)	734 (18.3)	768 (19.2)	758 (18.9)	
With Hypertension, n (%)	2208 (55.3)	2285 (57.2)	2214 (55.4)	2256 (56.5)	2169 (54.2)	0.22
With Diabetes, n (%)	775 (19.9)	721 (18.7)	712 (18.4)	647 (16.7)	663 (17.2)	0.09
BMI (kg/m^2)	29.4 ± 6.3	29.3 ± 6.1	28.9 ± 6.0	28.9 ±6.0	28.8 ±6.0	0.01
CAD history[4] (%)	404 (10.1)	383 (9.6)	379 (9.5)	416 (10.4)	431 (10.8)	0.25
Dietary fiber (g/d)	11.1 ± 5.2	14.5 ± 7.0	17.1 ± 8.0	18.2 ± 8.8	19.0 ± 10.2	<0.001
Saturated fat (g/d)	18.5 ± 9.8	19.8 ± 10.6	20.5 ± 10.8	21.2 ± 10.7	22.8 ± 11.3	<0.001
Polyunsaturated fat (g/d)	16.0 ± 9.1	17.6 ± 9.9	18.8 ± 10.2	19.8 ± 10.4	21.0 ± 11.1	<0.001
Omega-3 fats (g/d)	1.3 ± 0.7	1.5 ± 0.8	1.6 ± 0.9	1.7 ± 0.9	1.8 ± 0.9	<0.001
Vitamin C (mg/d)	62.7 ± 39.4	90.9 ± 41.5	119.8 ± 53.2	134.9 ± 79.3	137.9 ± 97.1	<0.001
Vitamin E (a-TE/d)	7.0 ± 3.7	8.9 ± 4.5	10.1 ± 5.2	10.6 ± 5.4	11.1 ± 5.5	<0.001
ß-carotene (µg/d)	2264 ± 1662	3125 ± 2327	3760 ± 2828	4161 ± 3283	4283 ± 3941	<0.001
Folate (µg/d)	238 ± 104	306 ± 126	357 ± 145	386 ± 157	416 ± 180	<0.001
% kcal from sweets	16.8 ± 11.3	14.8 ± 9.3	13.7 ± 8.9	14.2 ± 8.9	15.8 ± 10.0	<0.001
Whole grains (serving/d)	1.3 ± 1.3	1.5 ± 1.3	1.7 ± 1.4	1.8 ± 1.4	1.8 ± 1.5	<0.001
Sodium (mg/d)	1870 ± 730	2158 ± 988	2344 ± 1080	2432 ± 1076	2518 ± 1183	<0.001
Potassium (mg/d)	1901 ± 852	2370 ± 814	2777 ± 954	2990 ± 1036	3014 ± 1253	<0.001

[1] Values are means SD unless otherwise indicated. REGARDS, REasons for Geographic and Racial Differences in Stroke. Total flavonoid I is the sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

[2] Data were analyzed using ANOVA and chi-square tests for continuous and categorical variables, respectively.

[3] Stroke Belt includes Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee; Stroke Buckle includes coastal plains of Georgia, North Carolina and South Carolina, Non-Belt includes remaining area in lower 48 contiguous states. Regions are mutually exclusive.

[4] Cardiovascular disease history self-reported or ECG-detected at baseline

Table 4.2 Distribution of sex, self-reported race and region of residence by quintile of flavonoid subclass for 20,024 stroke-free participants in the Reasons for Geographic and Racial Differences in Stroke Study (REGARDS) 2003-2007[1]

	Q1	Q2	Q3	Q4	Q5	P
n	4004	4005	4006	4005	4004	
Anthocyanidins						
Intake (mg/d)	3.45 (≤ 4.89)	6.29 (4.90-7.86)	9.63 (7.87-11.83)	14.58 (11.84-18.49)	25.28 (≥ 18.50)	
Men	1852 (46.3)	1837 (45.9)	1738 (43.4)	1643 (41.0)	1698 (42.4)	<0.001
Black	1422 (35.5)	1463 (36.5)	1334 (33.3)	1326 (33.1)	1108 (27.7)	<0.001
Region[2]						
Stroke Belt	1459 (36.4)	1454 (36.3)	1383 (34.5)	1362 (34.0)	1233 (30.8)	<0.001
Stroke Buckle	916 (22.9)	857 (21.4)	887 (22.1)	849 (21.1)	864 (21.6)	
Non-Belt	1629 (40.7)	1694 (42.3)	1736 (43.4)	1794 (44.8)	1907 (47.6)	
Flavan-3-ols I[3]						
Intake (mg/d)	9.91 (≤ 15.21)	23.14 (15.22-35.00)	55.23 (35.01-115.61)	196.12 (115.62-269.09)	523.00 (≥ 269.10)	
Men	1743 (43.5)	1791 (44.7)	1745 (43.6)	1780 (44.4)	1714 (42.8)	0.42
Black	1716 (42.9)	1505 (37.6)	1440 (36.0)	1210 (30.2)	788 (19.7)	<0.001
Region[2]						
Stroke Belt	1339 (33.4)	1302 (32.5)	1299 (32.4)	1482 (37.0)	1473 (36.8)	<0.001
Stroke Buckle	739 (18.5)	758 (18.9)	810 (20.2)	923 (23.1)	1142 (26.1)	
Non-Belt	1927 (48.1)	1945 (48.6)	1895 (47.3)	1600 (40.0)	1390 (34.7)	
Flavan-3-ols II[4]						
Intake (mg/d)	7.96 (≤ 12.42)	17.75 (12.43-24.00)	35.38 (24.01-52.66)	87.12 (52.67-119.66)	221.00 (≥ 119.66)	
Men	1779 (24.8)	1754 (22.8)	1763 (22.7)	1760 (18.3)	1717 (11.5)	0.71
Black	1651 (41.2)	1518 (37.9)	1508 (37.7)	1215 (30.3)	767 (19.2)	<0.001
Region[2]						
Stroke Belt	1296 (32.4)	1265 (31.6)	1378 (34.4)	1482 (37.0)	1474 (36.8)	<0.001

Stroke Buckle	740 (18.5)	744 (18.6)	799 (20.0)	941(23.5)	1148 (28.7)	
Non-Belt	1968 (49.2)	1997 (49.9)	1828(45.6)	1581 (18.1)	1383 (34.5)	
Flavanones						
Intake (mg/d)	2.09 (≤ 3.95)	6.34 (3.96-9.98)	16.42 (9.99–23.72)	34.86 (23.73–47.96)	58.10 (≥ 47.97)	
Men	1694 (42.3)	1709 (42.7)	1702 (42.5)	1745 (43.6)	1923 (48.0)	<0.001
Black	950 (23.7)	1709 (30.9)	1702 (35.5)	1745 (38.5)	1923 (37.6)	<0.001
Region[2]						
Stroke Belt	1514 (37.8)	1452 (36.3)	1369 (34.2)	1298 (32.4)	1262 (31.5)	<0.001
Stroke Buckle	1007 (25.2)	903 (22.6)	869 (21.7)	825 (20.6)	768 (19.2)	
Non-Belt	1483 (37.0)	1650 (41.2)	1768 (44.1)	1882 (47.0)	1974 (49.3)	
Flavones						
Intake (mg/d)	0.32 (≤ 0.42)	0.53 (0.43–0.63)	0.76 (0.64–0.90)	1.12 (0.91-1.45)	2.09 (≥ 1.46)	
Men	1843 (46.0)	1851 (46.2)	1801 (45.0)	1720 (42.9)	1558 (38.9)	<0.001
Black	1835 (45.8)	1495 (37.3)	1286 (32.1)	1081 (27.0)	962 (24.0)	<0.001
Region[2]						
Stroke Belt	1503 (37.5)	1310 (32.7)	1335 (33.3)	1280 (32.0)	1467 (36.6)	<0.001
Stroke Buckle	798 (19.9)	873 (21.8)	871 (21.8)	888 (22.2)	942 (23.5)	
Non-Belt	1703 (42.5)	1822 (45.5)	1799 (44.9)	1838 (45.9)	1595 (39.8)	
Flavonols						
Intake (mg/d)	6.76 (≤ 8.98)	11.28 (8.99-13.53)	15.99 (13.54-18.96)	22.64 (18.97-27.66)	35.75 (≥ 27.67)	
Men	1658 (41.4)	1795 (44.8)	1819 (45.4)	1802 (45.0)	1699 (42.3)	<0.001
Black	1869 (46.7)	1544 (38.6)	1313 (32.8)	1122 (28.0)	811 (20.3)	<0.001
Region[2]						
Stroke Belt	1401 (35.0)	1318 (32.9)	1357 (33.9)	1325 (33.1)	1494 (37.3)	<0.001
Stroke Buckle	775 (19.4)	831 (20.8)	820 (20.5)	968 (24.2)	978 (24.4)	
Non-Belt	1829 (45.7)	1855 (46.3)	1829 (45.7)	1712 (42.8)	1532 (38.3)	
Isoflavones						
Intake (mg/d)	0.12 (≤ 0.18)	0.25 (0.19-0.33)	0.43 (0.34–0.56)	0.73 (0.57–1.02)	1.68 (≥ 1.03)	
Men	1485 (37.1)	1743 (43.5)	1819 (45.4)	1822 (45.5)	1904 (47.5)	<0.001
Black	1598 (39.9)	1427 (35.6)	1239 (30.9)	1090 (27.2)	1305 (32.6)	<0.001
Region[2]						

Stroke Belt	1508 (37.7)	1445 (36.1)	1393 (34.8)	1339 (33.4)	1210 (17.6)	<0.001
Stroke Buckle	856 (21.4)	910 (22.7)	914 (22.8)	886 (22.1)	806 (20.1)	
Non-Belt	1641 (41.0)	1649 (41.2)	1699 (42.4)	1779 (44.4)	1989 (49.7)	
Proanthocyanidins						
Intake (mg/d)	33.79 (≤ 46.84)	58.73 (46.85-70.91)	84.01 (70.92-98.75)	116.86 (98.76-141.33)	181.48 (≥ 141.33)	
Men	1645 (41.1)	1792 (44.7)	1697 (42.4)	1792 (45.0)	1847 (45.9)	<0.001
Black	1753 (43.8)	1415 (35.3)	1172 (29.3)	1101 (27.6)	1218 (30.3)	<0.001
Region[2]						
Stroke Belt	1408 (35.2)	1366 (34.1)	1373 (34.3)	1387 (34.8)	1361 (33.8)	<0.001
Stroke Buckle	870 (21.7)	921 (23.0)	893 (22.3)	845 (21.2)	843 (20.9)	
Non-Belt	1726 (43.1)	1718 (42.9)	1739 (43.4)	1752 (44.0)	1822 (45.3)	

[1] Data were analyzed using chi-squared tests

[2] Stroke Belt includes Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee; Stroke Buckle includes coastal plains of Georgia, North Carolina and South Carolina, Non-Belt includes remaining area in lower 48 contiguous states. Regions are mutually exclusive.

[3] Flavan-3-ols including thearubigins

[4] Flavan-3-ols excluding thearubigins

Table 4.3 Hazard Ratios and 95% CI for incident acute ischemic stroke (AIS) by quintile of flavonoid intake in the REGARDS study[1]

Quintile	Median Intake (range) mg/d	AIS *n*	Model I[4]	Model II[5]
Total Flavonoid I[2]				
1	34.31 (≤ 50.86)	112	1.00 (-)	1.00 (-)
2	66.59 (50.87-83.43)	116	1.00 (0.77, 1.30)	0.96 (0.74, 1.25)
3	102.97 (83.44-127.05)	106	0.91 (0.70, 1.18)	0.93 (0.71, 1.22)
4	156.94 (127.06-208.32)	82	0.69 (0.52, 0.92)	0.73 (0.55, 0.98)
5	296.84 (≥ 208.33)	108	0.93 (0.72, 1.22)	0.94 (0.71, 1.26)
P-trend[3]			0.40	0.62
Anthocyanidin				
1	3.45 (≤ 4.89)	126	1.00 (-)	1.00 (-)
2	6.29 (4.90-7.86)	98	0.76 (0.58, 0.98)	0.72 (0.55, 0.95)
3	9.63 (7.87-11.83)	93	0.73 (0.56, 0.95)	0.72 (0.54, 0.94)
4	14.58 (11.84-18.49)	91	0.70 (0.54, 0.92)	0.70 (0.53, 0.93)
5	25.28 (≥ 18.50)	116	0.90 (0.70-1.16)	0.93 (0.70, 1.23)
P-trend[3]			0.92	0.67
Flavan-3-ol				
1	7.96 (≤ 12.42)	119	1.00 (-)	1.00 (-)
2	17.75 (12.43-24.00)	92	0.75 (0.57, 0.98)	0.79 (0.60, 1.10)
3	35.38 (24.01-52.66)	120	0.97 (0.76, 1.26)	1.05 (0.81, 1.36)
4	87.12 (52.67-119.66)	86	0.69 (0.52, 0.91)	0.71 (0.54, 0.95)
5	221.00 (≥ 119.66)	107	0.88 (0.68, 1.14)	0.97 (0.73, 1.27)
P-trend[3]			0.77	0.93
Flavanone				
1	2.09 (≤ 3.95)	117	1.00 (-)	1.00 (-)
2	6.34 (3.96-9.98)	100	0.84 (0.64, 1.10)	0.88 (0.68, 1.16)
3	16.42 (9.99–23.72)	95	0.80 (0.61, 1.04)	0.82 (0.62, 1.08)
4	34.86 (23.73–47.96)	104	0.86 (0.66, 1.12)	0.80 (0.61, 1.05)
5	58.10 (≥ 47.97)	108	0.90 (0.69, 1.17)	0.73 (0.55, 0.95)
P-trend[3]			0.82	0.03
Flavone				
1	0.32 (≤ 0.42)	122	1.00 (-)	1.00 (-)
2	0.53 (0.43–0.63)	103	0.82 (0.63, 1.06)	0.88 (0.68, 1.15)
3	0.76 (0.64–0.90)	94	0.74 (0.57, 0.97)	0.87 (0.66, 1.14)
4	1.12 (0.91-1.45)	115	0.90 (0.69, 1.16)	1.08 (0.82, 1.42)
5	2.09 (≥ 1.46)	90	0.70 (0.53, 0.92)	0.94 (0.70, 1.26)
P-trend[3]			0.49	0.88
Flavonol				
1	6.76 (≤ 8.98)	108	1.00 (-)	1.00 (-)
2	11.28 (8.99-13.53)	129	1.17 (0.90, 1.51)	1.19 (0.92, 1.55)
3	15.99 (13.54-18.96)	92	0.80 (0.61, 1.06)	0.89 (0.67, 1.19)
4	22.64 (18.97-27.66)	91	0.80 (0.60, 1.05)	0.91 (0.68, 1.22)
5	35.75 (≥ 27.67)	104	0.92 (0.70, 1.21)	1.16 (0.86, 1.57)
P-trend[3]			0.72	0.79
Isoflavone				

1	0.12 (≤ 0.18)	119	1.00 (-)	1.00 (-)
2	0.25 (0.19-0.33)	113	0.93 (0.72, 1.20)	1.05 (0.81, 1.37)
3	0.43 (0.34–0.56)	101	0.82 (0.63, 1.07)	0.97 (0.74, 1.28)
4	0.73 (0.57–1.02)	97	0.79 (0.60, 1.03)	1.01 (0.76, 1.34)
5	1.68 (≥ 1.03)	94	0.76 (0.58, 0.99)	1.06 (0.79, 1.44)
P-trend[3]			0.37	0.11
Proanthocyanidin				
1	33.79 (≤ 46.84)	112	1.00 (-)	1.00 (-)
2	58.73 (46.85-70.91)	101	0.87 (0.67, 1.14)	0.91 (0.70, 1.20)
3	84.01 (70.92-98.75)	102	0.88 (0.67, 1.15)	0.92 (0.70, 1.22)
4	116.86 (98.76-141.33)	111	0.95 (0.73, 1.24)	1.04 (0.78, 1.37)
5	181.48 (≥ 141.33)	98	0.83 (0.64, 1.09)	0.89 (0.65, 1.23)
P-trend[3]			0.23	0.80
Total Flavonoid II[6]				
1	79.84 (≤ 109.58)	120	1.00 (-)	1.00 (-)
2	139.34 (109.59-168.63)	94	0.92 (0.75, 1.29)	0.74 (0.56, 0.97)
3	200.21 (168.64-237.57)	108	0.95 (0.73, 1.24)	0.83 (0.63, 1.09)
4	284.20 (237.58-346.90)	113	0.77 (0.57, 1.01)	0.87 (0.66, 1.15)
5	452.53 (≥ 346.91)	89	0.92 (0.71, 1.21)	0.73 (0.65, 1.01)
P-trend[3]			0.08	0.19

1. Hazard ratios and 95% CI estimated using Cox proportional hazards models. AIS, acute ischemic stroke, REGARDS, REasons for Geographic and Racial Disparities in Stroke

2. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

3. Test for trend using median values for each quintile, modeled as a continuous variable.

4. Unadjusted model

5. Adjusted for age, energy, sex, race, region of residence, educational, income, exercise, smoking status, self-report of CAD at baseline, age x race interaction term

6. Sum of total flavonoid I and proanthocyanidin intakes

Table 4.4 Hazard ratios and 95% confidence interval for incident acute ischemic stroke (AIS) by quintile of flavonoid intake for participants in the REGARDS[1] study, stratified by self-reported race.

		White (*n* = 13,365)			Black (*n* = 6659)	
Quintile	Median Intake (range) mg/d	AIS *n*	Model I[4]	Model II[5]	AIS *n*	Model I[4]	Model II[5]
Total Flavonoid I[2]							
1	34.31 (≤ 50.86)	69	1.00 (-)	1.00 (-)	41	1.00 (-)	1.00 (-)
2	66.59 (50.87-83.43)	66	0.90 (0.64, 1.26)	0.88 (0.63, 1.24)	45	1.10 (0.67, 1.56)	0.87 (0.56, 1.34)
3	102.97 (83.44-127.05)	71	0.92 (0.66, 1.28)	0.90 (0.64, 1.27)	38	1.00 (0.64, 1.52)	0.99 (0.63, 1.55)
4	156.94 (127.06-208.32)	60	0.72 (0.51, 1.01)	0.67 (0.47, 0.96)	29	0.88 (0.45, 1.21)	0.91 (0.57, 1.48)
5	296.84 (≥ 208.33)	84	0.91 (0.66, 1.26)	0.96 (0.69, 1.34)	21	0.92 (0.62, 1.72)	0.71 (0.39, 1.29)
P-trend[3]			0.63	0.98		0.50	0.34
Anthocyanidin							
1	3.45 (≤ 4.89)	75	1.00 (-)	1.00 (-)	51	1.00 (-)	1.00 (-)
2	6.29 (4.90-7.86)	67	0.88 (0.63, 1.22)	0.82 (0.59, 1.15)	31	0.58 (0.37, 0.90)	0.58 (0.37, 0.91)
3	9.63 (7.87-11.83)	61	0.78 (0.55, 1.09)	0.75 (0.55, 1.10)	32	0.66 (0.43, 1.03)	0.66 (0.42, 1.05)
4	14.58 (11.84-18.49)	64	0.80 (0.58, 1.21)	0.78 (0.53, 1.07)	27	0.55 (0.35, 0.88)	0.56 (0.34, 0.92)
5	25.28 (≥ 18.50)	83	0.97 (0.71, 1.32)	0.98 (0.69, 1.38)	33	0.83 (0.53, 1.28)	0.83 (0.51, 1.36)
P-trend[3]			0.86	0.59		0.70	0.88
Flavan-3-ol							
1	7.96 (≤ 12.42)	71	1.00 (-)	1.00 (-)	48	1.00 (-)	1.00 (-)
2	17.75 (12.43-24.00)	62	0.79 (0.56, 1.11)	0.97 (0.70, 1.34)	30	0.69 (0.44, 1.09)	0.71 (0.44, 1.13)
3	35.38 (24.01-52.66)	71	0.86 (0.62, 1.19)	0.91 (0.48, 0.96)	49	1.20 (0.81, 1.79)	1.33 (0.88, 1.99)
4	87.12 (52.67-119.66)	59	0.65 (0.65, 0.92)	0.68 (0.65, 1.27)	27	0.77 (0.48, 1.24)	0.79 (0.49, 1.28)
5	221.00 (≥ 119.66)	87	0.86 (0.63, 1.17)	0.97 (0.70, 1.34)	20	0.90 (0.53, 1.52)	0.90 (0.52, 1.53)
P-trend[3]			0.77	0.60		0.75	0.69
Flavanone							
1	2.09 (≤ 3.95)	88	1.00 (-)	1.00 (-)	29	1.00 (-)	1.00 (-)
2	6.34 (3.96-9.98)	61	0.74 (0.55, 1.03)	0.78 (0.56, 1.09)	39	1.03 (0.64, 1.66)	1.10 (0.68, 1.78)
3	16.42 (9.99–23.72)	67	0.88 (0.62, 1.21)	0.90 (0.66, 1.25)	28	0.62 (0.37, 1.05)	0.67 (0.40, 1.13)
4	34.86 (23.73–47.96)	66	0.89 (0.54, 1.23)	0.82 (0.59, 1.14)	38	0.77 (0.47, 1.25)	0.78 (0.48, 1.26)
5	58.10 (≥ 47.97)	68	0.91 (0.46, 1.25)	0.70 (0.50, 0.97)	40	0.84 (0.52, 1.36)	0.77 (0.47, 1.25)
P-trend[3]			0.87	0.07		0.12	0.19
Flavone							

1	0.32 (≤ 0.42)	77	1.00 (-)	1.00 (-)	45	1.00 (-)	1.00 (-)
2	0.53 (0.43–0.63)	65	0.71 (0.51, 0.98)	0.79 (0.56, 1.10)	38	1.02 (0.66, 1.57)	1.06 (0.68, 1.64)
3	0.76 (0.64–0.90)	60	0.60 (0.43, 0.83)	0.72 (0.51, 1.02)	34	1.06 (0.68, 1.66)	1.18 (0.74, 1.87)
4	1.12 (0.91-1.45)	76	0.69 (0.50, 0.95)	0.87 (0.62, 1.21)	39	1.46 (0.95, 2.24)	1.66 (1.04, 2.63)
5	2.09 (≥ 1.46)	72	0.64 (0.46, 0.88)	0.89 (0.63, 1.25)	18	0.73 (0.42, 1.34)	0.92 (0.51, 1.63)
P-trend[3]			0.07	0.88		0.50	0.95
Flavonol							
1	6.76 (≤ 8.98)	64	1.00 (-)	1.00 (-)	44	1.00 (-)	1.00 (-)
2	11.28 (8.99-13.53)	80	1.10 (0.78, 1.50)	1.11 (0.79, 1.55)	49	1.30 (0.87, 1.96)	1.32 (0.87, 2.00)
3	15.99 (13.54-18.96)	60	0.71 (0.50, 1.00)	0.79 (0.55, 1.14)	32	0.99 (0.63, 1.54)	1.07 (0.67, 1.72)
4	22.64 (18.97-27.66)	65	0.72 (0.51, 1.01)	0.84 (0.58, 1.20)	26	0.95 (0.58, 1.56)	1.03 (0.62, 1.72)
5	35.75 (≥ 27.67)	81	0.82 (0.59, 1.14)	1.08 (0.75, 1.55)	23	1.18 (0.71, 1.95)	1.32 (0.75, 2.34)
P-trend[3]			0.11	0.79		0.95	0.48
Isoflavone							
1	0.12 (≤ 0.18)	68	1.00 (-)	1.00 (-)	51	1.00 (-)	1.00 (-)
2	0.25 (0.19-0.33)	72	0.96 (0.69, 1.34)	1.12 (0.80, 1.57)	41	0.89 (0.59, 1.34)	0.95 (0.63, 1.45)
3	0.43 (0.34–0.56)	68	0.84 (0.60, 1.18)	1.04 (0.73, 1.46)	33	0.81 (0.52, 1.25)	0.89 (0.57, 1.40)
4	0.73 (0.57–1.02)	74	0.87 (0.63, 1.21)	1.21 (0.85, 1.70)	23	0.65 (0.40, 1.06)	0.68 (0.40, 1.16)
5	1.68 (≥ 1.03)	68	0.85 (0.61, 1.19)	1.34 (0.93, 1.94)	26	0.61 (0.38, 0.98)	0.66 (0.39, 1.14)
P-trend[3]			0.37	0.11		0.03	0.11
Proanthocyanidin							
1	33.79 (≤ 46.84)	67	1.00 (-)	1.00 (-)	45	1.00 (-)	1.00 (-)
2	58.73 (46.85-70.91)	64	0.80 (0.57, 1.14)	0.85 (0.60, 1.21)	37	1.00 (0.65, 1.56)	1.00 (0.65, 1.56)
3	84.01 (70.92-98.75)	76	0.88 (0.63, 1.13)	0.94 (0.67, 1.32)	26	0.83 (0.51, 1.35)	0.83 (0.50, 1.36)
4	116.86 (98.76-141.33)	72	0.81 (0.58, 1.22)	0.89 (0.63, 1.27)	39	1.33 (0.87, 2.04)	1.40 (0.88, 2.23)
5	181.48 (≥ 141.33)	71	0.81 (0.58, 1.13)	0.91 (0.62, 1.33)	27	0.83 (0.51, 1.34)	0.83 (0.47, 1.47)
P-trend[3]			0.37	0.80		0.40	0.84
Total Flavonoid II[6]							
1	79.84 (≤ 109.58)	74	1.00 (-)	1.00 (-)	45	1.00 (-)	1.00 (-)
2	139.34 (109.59-168.63)	56	0.70 (0.49, 0.98)	0.67 (0.47, 0.95)	38	0.86 (0.56, 1.33)	0.93 (0.60, 1.44)
3	200.21 (168.64-237.57)	72	0.78 (0.57, 1.08)	0.75 (0.54, 1.17)	42	1.00 (0.64, 1.54)	1.10 (0.71, 1.71)
4	284.20 (237.58-346.90)	79	0.85 (0.62, 1.17)	0.83 (0.60, 1.17)	27	0.96 (0.62, 1.49)	0.80 (0.48, 1.33)
5	452.53 (≥ 346.91)	69	0.68 (0.48, 0.98)	0.72 (0.50, 1.02)	22	0.76 (0.45, 1.28)	0.92 (0.53, 1.61)
P-trend[3]			0.13	0.35		0.42	0.33

1. Hazard ratios and 95% confidence intervals estimated using Cox proportional hazards models. AIS, acute ischemic stroke, REGARDS, REasons for Geographic and Racial Disparities in Stroke

2. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

3. Test for trend using median values for each quintile, modeled as a continuous variable.

4. Unadjusted model

5. Adjusted for age, energy, sex, race, region of residence, educational, income, exercise, smoking status, self-report of CAD at baseline, age x race interaction term

6. Sum of total flavonoid I and proanthocyanidin intakes

Table 4.5. Hazard ratios and 95% confidence interval for incident acute ischemic stroke (AIS) by quintile of flavonoid intake for participants in the REGARDS[1] study, stratified by residence in Stroke Belt.

		Stroke Belt/Stroke Buckle (*n* = 11,267)			Not Stroke Belt (*n* = 8,757)		
Quintile	Median Intake (range) mg/d	AIS *n*	Model I[4]	Model II[5]	AIS *n*	Model I[4]	Model II[5]
Total Flavonoid							
I[2]							
1	37.93 (≤ 57.95)	60	1.00 (-)	1.00 (-)	50	1.00 (-)	1.00 (-)
2	77.81 (57.96-101.36)	55	0.91 (0.63, 1.31)	0.90 (0.62, 1.31)	56	1.05 (0.72, 1.54)	1.02 (0.70, 1.51)
3	131.97 (101.37-178.22)	55	0.91 (0.63, 1.31)	0.88 (0.61, 1.29)	54	1.00 (0.68, 1.47)	0.96 (0.65, 1.43)
4	258.92 (179.23-356.51)	47	0.70 (0.48, 1.02)	0.67 (0.45, 0.99)	42	0.86 (0.57, 1.29)	0.81 (0.53, 1.24)
5	594.39 (≥ 356.52)	70	0.96 (0.68, 1.35)	0.99 (0.69, 1.43)	35	0.85 (0.55, 1.31)	0.84 (0.54, 1.33)
P-trend[3]			0.30	0.88		0.75	0.65
Anthocyanidin							
1	3.45 (≤ 4.89)	73	1.00 (-)	1.00 (-)	53	1.00 (-)	1.00 (-)
2	6.29 (4.90-7.86)	58	0.81 (0.57, 1.14)	0.77 (0.54, 1.09)	40	0.69 (0.46, 1.04)	0.67 (0.44, 1.01)
3	9.63 (7.87-11.83)	45	0.64 (0.44, 0.93)	0.64 (0.43, 0.93)	48	0.84 (0.57, 1.23)	0.81 (0.54, 1.01)
4	14.58 (11.84-18.49)	45	0.64 (0.44, 0.93)	0.62 (0.42, 0.91)	46	0.53 (0.53, 1.16)	0.79 (0.52, 1.21)
5	25.28 (≥ 18.50)	66	1.00 (0.71, 1.40)	0.98 (0.98, 1.43)	50	0.54 (0.54, 1.17)	0.85 (0.55, 1.30)
P-trend[3]			0.71	0.59		0.41	0.61
Flavan-3-ol							
1	7.96 (≤ 12.42)	61	1.00 (-)	1.00 (-)	58	1.00 (-)	1.00 (-)
2	17.75 (12.43-24.00)	45	0.73 (0.49, 1.07)	0.75 (0.52, 1.10)	47	0.79 (0.53, 1.15)	0.84 (0.57, 1.23)
3	35.38 (24.01-52.66)	61	0.94 (0.66, 1.34)	0.99 (0.70, 1.45)	59	1.02 (0.71, 1.46)	1.09 (0.75, 1.58)
4	87.12 (52.67-119.66)	48	0.65 (0.44, 0.94)	0.69 (0.45, 0.99)	38	0.76 (0.50, 1.14)	0.77 (0.50, 1.17)
5	221.00 (≥ 119.66)	72	0.90 (0.64, 1.27)	1.00 (0.70, 1.43)	35	0.82 (0.54, 1.24)	0.88 (0.57, 1.36)
P-trend[3]			0.96	0.53		0.60	0.60
Flavanone							
1	2.09 (≤ 3.95)	78	1.00 (-)	1.00 (-)	39	1.00 (-)	1.00 (-)
2	6.34 (3.96-9.98)	58	0.77 (0.55, 1.09)	0.81 (0.57, 1.13)	42	0.96 (0.62, 1.49)	1.03 (0.67, 1.60)
3	16.42 (9.99–23.72)	51	0.72 (0.51, 1.02)	0.71 (0.50, 1.02)	44	0.94 (0.61, 1.48)	1.02 (0.66, 1.57)
4	34.86 (23.73–47.96)	43	0.63 (0.43, 0.91)	0.58 (0.84, 0.84)	61	1.21 (0.81, 1.81)	1.16 (0.77, 1.75)
5	58.10 (≥ 47.97)	57	0.87 (0.62, 1.23)	0.69 (0.49, 0.99)	51	0.98 (0.65, 1.48)	0.82 (0.55 1.26)
P-trend[3]			0.50	0.04		0.71	0.35

Flavone							
1	0.32 (≤ 0.42)	66	1.00 (-)	1.00 (-)		1.00 (-)	1.00 (-)
2	0.53 (0.43–0.63)	60	0.93 (0.65, 1.31)	1.01 (0.71, 1.44)	56	0.70 (0.47, 1.04)	0.75 (0.50, 1.12)
3	0.76 (0.64–0.90)	43	0.65 (0.44, 0.95)	0.78 (0.53, 1.17)	43	0.84 (0.58, 1.23)	0.95 (0.64, 1.40)
4	1.12 (0.91-1.45)	63	0.96 (0.68, 1.35)	1.14 (0.79, 1.65)	51	0.83 (0.57, 1.21)	1.01 (0.67, 1.51)
5	2.09 (≥ 1.46)	55	0.76 (0.53, 1.08)	0.99 (0.68, 1.47)	52	0.63 (0.41, 0.96)	0.86 (0.55, 1.36)
P-trend[3]			0.25	0.78	35	0.10	0.95
Flavonol							
1	6.76 (≤ 8.98)	59	1.00 (-)	1.00 (-)	49	1.00 (-)	1.00 (-)
2	11.28 (8.99-13.53)	65	1.10 (0.77, 1.55)	1.15 (0.80, 1.64)	64	1.26 (0.87, 1.82)	1.23 (0.84, 1.80)
3	15.99 (13.54-18.96)	47	0.74 (0.51, 1.10)	0.83 (0.56, 1.24)	45	0.88 (0.59, 1.31)	0.97 (0.63, 1.48)
4	22.64 (18.97-27.66)	51	0.77 (0.53, 1.11)	0.86 (0.58, 1.29)	40	0.84 (0.55, 1.27)	0.96 (0.61, 1.49)
5	35.75 (≥ 27.67)	65	0.92 (0.65, 1.31)	1.16 (0.78, 1.72)	39	0.91 (0.60, 1.38)	1.14 (0.71, 1.82)
P-trend[3]			0.43	0.79		0.23	0.93
Isoflavone							
1	0.12 (≤ 0.18)	68	1.00 (-)	1.00 (-)	51	1.00 (-)	1.00 (-)
2	0.25 (0.19-0.33)	64	0.92 (0.65, 1.29)	1.07 (0.76, 1.51)	49	0.93 (0.63, 1.34)	1.04 (0.70, 1.55)
3	0.43 (0.34–0.56)	53	0.78 (0.54, 1.10)	0.93 (0.64, 1.34)	48	0.88 (0.60, 1.31)	1.04 (0.69, 1.56)
4	0.73 (0.57–1.02)	55	0.83 (0.58, 1.18)	1.05 (0.72, 1.53)	42	0.74 (0.49, 1.11)	0.96 (0.62, 1.48)
5	1.68 (≥ 1.03)	47	0.78 (0.54, 1.13)	1.06 (0.70, 1.60)	47	0.73 (0.49, 1.08)	1.08 (0.69, 1.68)
P-trend[3]			0.37	0.11		0.10	0.78
Proanthocyanidin							
1	33.79 (≤ 46.84)	68	1.00 (-)	1.00 (-)	44	1.00 (-)	1.00 (-)
2	58.73 (46.85-70.91)	48	0.68 (0.47, 0.98)	0.71 (0.49, 1.03)	53	1.18 (0.79, 1.76)	1.21 (0.81, 1.82)
3	84.01 (70.92-98.75)	60	0.86 (0.61, 1.21)	0.90 (0.63, 1.29)	52	0.91 (0.59, 1.38)	0.95 (0.61, 1.47)
4	116.86 (98.76-141.33)	56	0.79 (0.56, 1.13)	0.84 (0.57, 1.23)	55	1.20 (0.81, 1.78)	1.34 (0.88, 2.06)
5	181.48 (≥ 141.33)	55	0.79 (0.56, 1.13)	0.82 (0.54, 1.25)	43	0.89 (0.59, 1.36)	0.98 (0.61, 1.60)
P-trend[3]			0.47	0.80		0.51	0.92
Total Flavonoid II[6]							
1	86.20 (≤ 120.81)	67	1.00 (-)	1.00 (-)	53	1.00 (-)	1.00 (-)
2	157.53 (120.82-195.94)	50	0.75 (0.52, 1.08)	0.73 (0.51, 1.06)	44	0.76 (0.51, 1.14)	0.74 (0.50, 1.12)
3	240.68 (195.95-300.18)	53	0.73 (0.51, 1.05)	0.70 (0.48, 1.01)	55	1.01 (0.69, 1.47)	1.00 (0.67, 1.48)
4	371.9 (300.19-497.94)	59	0.81 (0.57, 0.15)	0.78 (0.54, 1.14)	54	0.99 (0.68, 1.45)	0.97 (0.65, 1.47)
5	719.01 (≥ 497.95)	58	0.74 (0.52, 1.06)	0.77 (0.51, 1.12)	31	0.65 (0.42, 1.01)	0.66 (0.40, 1.08)
P-trend[3]			0.27	0.43		0.16	0.26

1. Hazard ratios and 95% confidence intervals estimated using Cox proportional hazards models. AIS, acute ischemic stroke, REGARDS, REasons for Geographic and Racial Disparities in Stroke

2. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

3. Test for trend using median values for each quintile, modeled as a continuous variable.

4. Unadjusted model

5. Adjusted for age, energy, sex, race, region of residence, educational, income, exercise, smoking status, self-report of CAD at baseline, age x race interaction term

6. Sum of total flavonoid I and proanthocyanidin intakes

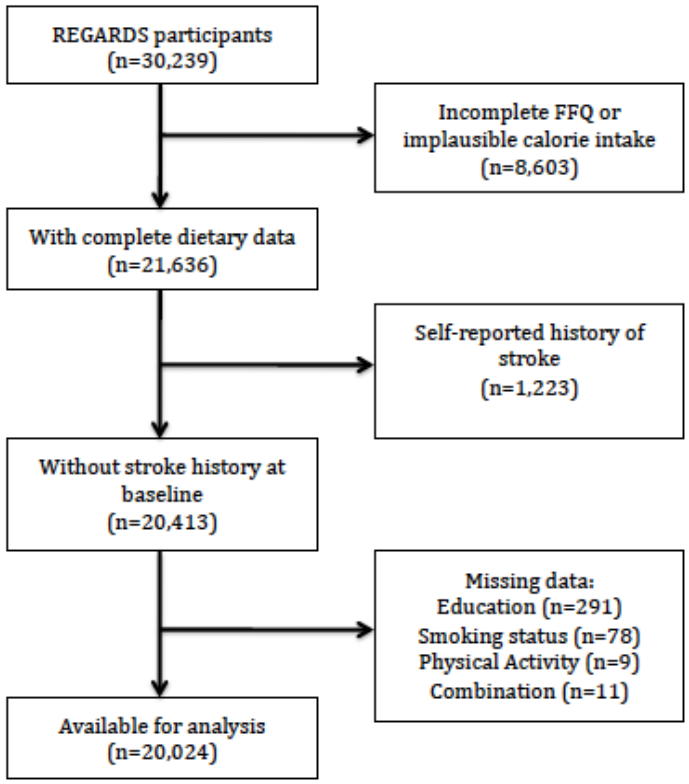

Figure 4.1 Study flow diagram for the inclusion of participants free from history of stroke at baseline enrollment in the REGARDS study. FFQ, food frequency questionnaire; REGARDS, REasons for Geographic and Racial Differences in Stroke.

Table S.4.1. Flavonoid subclasses, compounds and common food sources included in the US Department of Agriculture flavonoid databases

USDA Database	Flavonoid subclass (common food sources)	Flavonoid Compounds
Provisional Flavonoid Addendum[1]	Anthocyanidins (blueberries, strawberries, red wine)	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin
	Flavan_3_ols [3] (apples, tea, chocolate)	Catechin, Epicatechin, Epicatechin 3-gallate, Epigallocatechin, Epigallocatechin 3-gallate, Gallocatechin, Theaflavin, Theaflavin 3-gallate, Theaflavin 3'-gallate, Theaflavin 3,3'-digallate, Thearubigins
	Flavanones (citrus fruit and juices)	Eriodictyol, Hesperetin, Naringenin
	Flavones (celery, parsley, green pepper)	Apigenin, Luteolin
	Flavonols (onion, apple, broccoli)	Isorhamnetin, Kaempferol, Myricetin, Quercetin
	Isoflavones (tofu, soymilk, soy-based foods)	Daidzein, Genistein, Glycitein
Proanthocyanidin	Proanthocyanidins (apples, chocolate, red wine)	Monomers, dimers, trimers, 4-6mers, 7-10mers, polymers

1. Sebastian RS, Goldman JD, Martin CL, Steinfeldt LC, Enns CW, Moshfegh AJ. Flavonoid Values for USDA Survey Foods and Beverages 2007-2008: Provisional Flavonoid Addendum to the USDA Food and Nutrient Database for Dietary Studies, 4.1, and Flavonoid Intake Files from What We Eat in America (WWEIA), National Health and Nutrition Examination Survey (NHANES) 2007-2008. In: U.S. Department of Agriculture ARS, Food Surveys Research Group.,ed. Beltsville, MD, 2014.

2. U.S. Department of Agriculture Agricultural Research Service. USDA database for the proanthocyanidin content of selected foods. Beltsville, MD: Agricultural Research Service Nutrient Data Laboratory, 2004.

3. Thearubigins are included in the USDA Provisional Flavonoid Addendum. In this analysis, thearubigins were excluded from the main analysis because there is no consensus on structures and analytical methods. Thearubigins were included in sensitivity analyses.

Table S.4.2. Major food sources of flavonoids by Block98 Food Frequency Questionnaire item for participants without a history of stroke at baseline in the REGARDS[1] study

Flavonoid subclass	Men		Women	
	Food source	% [2]	Food source	% [2]
Anthocyanidins	Wine	27	Wine	17
	100% fruit juice, not orange	16	Berries, raw	16
	Berries, raw	14	100% fruit juice, not orange	13
	Cole slaw, cabbage	8	Yogurt	11
	Vegetable stew	8	Cole slaw, cabbage	7
Flavan-3-ols	Tea	83 (92)	Tea	84 (93)
	Wine	3 (1)	Apples and pears, raw	3 (1)
	Apples and pears, raw	3 (1)	100% fruit juice, not orange	2 (1)
	Bananas, raw	3 (1)	Bananas, raw	2 (1)
	100% fruit juice, not orange	2 (1)	Canned fruits	2 (0.9)
Flavanones	Orange juice	70	Orange juice	64
	Orange, raw	19	Orange, raw	24
	Grapefruit, raw	7	Grapefruit, raw	8
	100% fruit juice, not orange	2	100% fruit juice, not orange	2
	Cocktails	1	Cocktails	0.4
Flavones	Other vegetables[3]	38	Other vegetables[3]	39
	Salad greens	11	Salad greens	11
	Tea	6	Tea	6
	Spinach	3	Spinach	4
	Cantaloupe, raw	3	Cantaloupe, raw	3
Flavonols	Tea	31	Tea	32
	Salad greens	12	Salad greens	13
	Other vegetables[2]	8	Other vegetables[2]	9
	Apples and pears, raw	4	Apples and pears, raw	5
	Spinach, raw	2	Spinach, raw	3

Table S.4.2. Continued

Proanthocyanidins	Cakes[4]	19	Apples and pears, raw	19
	Apples and pears, raw	19	Cakes[4]	15
	Pies and cobblers[5]	13	Tea	10
	Tea	10	Pies and cobblers[5]	9
	Canned fruit	8	Canned fruit	9
Isoflavones	Soy milk	35	Soy milk	51
	Nuts including peanuts	20	Tofu	15
	Tofu	16	Nuts including peanuts	14
	Pastries	11	Pastries	7
	Other vegetables[3]	5	Other vegetables[3]	5
Total Flavonoids I [6]	Tea	51 (79)	Tea	48 (72)
	Orange juice	10 (9)	Orange juice	9 (8)
	Oranges, raw	4 (3)	Oranges, raw	4 (3)
	Wine	4 (2)	Wine	4 (2)
	100% fruit juice, not orange	3 (2)	100% fruit juice, not orange	3 (2)
Total Flavonoids II [7]	Tea	33 (61)	Tea	30 (53)
	Apples and pears, raw	9 (7)	Apples and pears, raw	9 (7)
	Cakes[4]	7 (6)	Cakes[4]	6 (4)
	Orange juice	5 (5)	Canned fruit	3 (3)
	Pies and cobblers[5]	5 (4)	Orange juice	3 (3)

1. REasons for Geographic and Racial Differences in Stroke study

2. Percent contributed to subclass (when including thearubigins)

3. Flavones primarily from squashes, peppers, celery; Flavonols primarily from asparagus, onions, peppers; Isoflavones primarily from soybeans.

4. Includes fruit, chocolate, and spice cakes; important sources of flavonoids include fruits, chocolate, and cinnamon.

5. Includes fruit cobblers, chocolate pies and turnovers; important sources of flavonoids include fruits, chocolate, and cinnamon.

6. Total flavonoid I intake includes anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols and isoflavones.

7. Total flavonoid II intake includes total flavonoid I plus proanthocyanidins.

Table S.4.3. Hazard ratios and 95% confidence interval for incident acute ischemic stroke (AIS) by quintile of flavonoid intake for participants in the REasons for Geographic and Racial Differences in Stroke, stratified by sex.

Quintile	Median Intake (range) mg/d	Men (*n*=8773) AIS *n*	Men (*n*=8773) Model I[3]	Men (*n*=8773) Model II[4]	Women (*n*=11251) AIS *n*	Women (*n*=11251) Model I[3]	Women (*n*=11251) Model II[4]
Total Flavonoid I[1]							
1	34.31 (≤ 50.86)	56	1.00 (-)	1.00 (-)	54	1.00 (-)	1.00 (-)
2	66.59 (50.87-83.43)	63	1.06 (0.74, 1.53)	1.05 (0.73, 1.52)	48	0.94 (0.59, 1.29)	0.86 (0.58, 1.28)
3	102.97 (83.44-127.05)	55	0.91 (0.63, 1.33)	0.91 (0.62, 1.34)	54	0.78 (0.67, 1.43)	0.94 (0.64, 1.38)
4	156.94 (127.06-208.32)	47	0.74 (0.52, 1.11)	0.74 (0.49, 1.11)	42	0.64 (0.51, 0.14)	0.73 (0.48, 1.10)
5	296.84 (≥ 208.33)	53	0.92 (0.63, 1.34)	0.97 (0.66, 1.44)	52	0.95 (0.64, 1.36)	0.92 (0.61, 1.38)
P-trend[2]			0.69	0.74		0.10	0.86
Anthocyanidin							
1	3.45 (≤ 4.89)	66	1.00 (-)	1.00 (-)	60	1.00 (-)	1.00 (-)
2	6.29 (4.90-7.86)	52	0.78 (0.54, 1.12)	0.78 (0.54, 1.12)	46	0.74 (0.50, 1.08)	0.67 (0.45, 0.99)
3	9.63 (7.87-11.83)	53	0.85 (0.53, 1.21)	0.84 (0.58, 1.22)	40	0.62 (0.42, 0.92)	0.59 (0.39, 0.90)
4	14.58 (11.84-18.49)	50	0.84 (0.59, 1.21)	0.84 (0.57, 1.24)	41	0.60 (0.40, 0.89)	0.57 (0.38, 0.87)
5	25.28 (≥ 18.50)	53	0.85 (0.60, 1.23)	0.88 (0.59, 1.32)	63	0.96 (0.68, 1.37)	0.96 (0.65, 1.43)
P-trend[2]			0.67	0.90		0.65	0.42
Flavan-3-ol							
1	7.96 (≤ 12.42)	60	1.00 (-)	1.00 (-)	59	1.00 (-)	1.00 (-)
2	17.75 (12.43-24.00)	51	0.81 (0.56, 1.18)	0.87 (0.60, 1.28)	41	0.69 (0.46, 1.03)	0.71 (0.49, 1.06)
3	35.38 (24.01-52.66)	69	1.11 (0.78, 1.56)	1.23 (0.86, 1.76)	51	0.84 (0.58, 1.22)	0.88 (0.60, 1.29)
4	87.12 (52.67-119.66)	42	0.66 (0.44, 0.98)	0.71 (0.47, 1.06)	44	0.72 (0.49, 1.07)	0.71 (0.48, 1.07)
5	221.00 (≥ 119.66)	52	0.86 (0.59, 1.24)	0.97 (0.66, 1.42)	55	0.90 (0.62, 1.30)	0.97 (0.66, 1.43)
P-trend[2]			0.37	0.64		0.78	0.44
Flavanone							
1	2.09 (≤ 3.95)	54	1.00 (-)	1.00 (-)	63	1.00 (-)	1.00 (-)
2	6.34 (3.96-9.98)	57	1.05 (0.73, 1.53)	1.12 (0.77, 1.63)	43	0.66 (0.45, 1.15)	0.67 (0.46, 1.00)
3	16.42 (9.99–23.72)	46	0.85 (0.57, 1.26)	0.90 (0.61, 1.34)	49	0.75 (0.51, 1.07)	0.74 (0.51, 1.08)
4	34.86 (23.73–47.96)	56	0.99 (0.68, 1.44)	0.99 (0.68, 1.45)	48	0.73 (0.50, 1.08)	0.64 (0.44, 0.94)
5	58.10 (≥ 47.97)	61	0.99 (0.68, 1.42)	0.86 (0.59, 1.26)	47	0.79 (0.54, 1.15)	0.60 (0.40, 0.88)
P-trend[2]			0.96	0.30		0.86	0.03

Table S.4.3. continued

Flavone							
1	0.32 (≤ 0.42)	72	1.00 (-)	1.00 (-)	50	1.00 (-)	1.00 (-)
2	0.53 (0.43–0.63)	54	0.72 (0.51, 1.02)	0.78 (0.55, 1.12)	49	0.96 (0.65, 1.42)	1.02 (0.69, 1.53)
3	0.76 (0.64–0.90)	53	0.72 (0.51, 1.03)	0.83 (0.88, 1.71)	41	0.78 (0.52, 1.18)	0.92 (0.60, 1.41)
4	1.12 (0.91-1.45)	50	0.70 (0.49, 1.00)	0.84 (0.57, 1.24)	65	1.19 (0.82, 1.72)	1.39 (0.94, 2.06)
5	2.09 (≥ 1.46)	45	0.70 (0.48, 1.01)	0.90 (0.60, 1.35)	45	0.76 (0.51, 1.14)	1.00 (0.65, 1.54)
P-trend[2]			0.30	0.98		0.61	0.84
Flavonol							
1	6.76 (≤ 8.98)	56	1.00 (-)	1.00 (-)	52	1.00 (-)	1.00 (-)
2	11.28 (8.99-13.53)	75	1.22 (0.86, 1.73)	1.26 (0.88, 1.79)	54	1.07 (0.71, 1.54)	1.12 (0.76, 1.64)
3	15.99 (13.54-18.96)	43	0.65 (0.44, 0.97)	0.74 (0.49, 1.11)	49	0.97 (0.65, 1.43)	1.08 (0.72, 1.62)
4	22.64 (18.97-27.66)	44	0.68 (0.46, 1.00)	0.78 (0.51, 1.18)	47	0.93 (0.62, 1.37)	1.07 (0.70, 1.62)
5	35.75 (≥ 27.67)	56	0.93 (0.64, 1.35)	1.20 (0.79, 1.82)	48	0.90 (0.61, 1.34)	1.10 (0.72, 1.71)
P-trend[2]			0.35	0.73		0.44	0.78
Isoflavone							
1	0.12 (≤ 0.18)	47	1.00 (-)	1.00 (-)	54	1.00 (-)	1.00 (-)
2	0.25 (0.19-0.33)	58	1.04 (0.71, 1.52)	1.21 (0.82, 1.79)	54	0.83 (0.58, 1.18)	0.67 (0.46, 0.97)
3	0.43 (0.34–0.56)	62	1.05 (0.72, 1.54)	1.38 (0.93, 2.03)	52	0.60 (0.41, 0.89)	0.72 (0.49, 1.07)
4	0.73 (0.57–1.02)	53	0.89 (0.73, 1.32)	1.24 (0.82, 1.86)	49	0.69 (0.47, 1.00)	0.72 (0.47, 1.19)
5	1.68 (≥ 1.03)	54	0.86 (0.58, 1.27)	1.37 (0.89, 2.10)	41	0.65 (0.44, 0.95)	0.84 (0.56, 1.26)
P-trend[2]			0.25	0.35		0.06	0.85
Proanthocyanidin							
1	33.79 (≤ 46.84)	58	1.00 (-)	1.00 (-)	72	1.00 (-)	1.00 (-)
2	58.73 (46.85-70.91)	47	0.72 (0.49, 1.06)	0.77 (0.52, 1.14)	55	1.03 (0.71, 1.51)	0.68 (0.46, 1.02)
3	84.01 (70.92-98.75)	50	0.82 (0.56, 1.19)	0.90 (0.61, 1.33)	39	0.94 (0.64, 1.37)	0.89 (0.60, 1.30)
4	116.86 (98.76-141.33)	62	0.94 (0.66, 1.35)	1.07 (0.73, 1.57)	44	0.94 (0.64, 1.38)	0.87 (0.58, 1.29)
5	181.48 (≥ 141.33)	57	0.72 (0.59, 1.22)	1.00 (0.65, 1.54)	40	0.78 (0.52, 1.17)	0.71 (0.46, 1.11)
P-trend[2]			0.97	0.91		0.17	0.21

Table S.4.3. Continued

Total Flavonoid II[5]							
1	79.84 (≤ 109.58)	61	1.00 (-)	1.00 (-)	59	1.00 (-)	1.00 (-)
2	139.34 (109.59-168.63)	53	0.79 (0.55, 1.15)	0.78 (0.54, 1.14)	41	0.69 (0.47, 1.03)	0.75 (0.51, 1.11)
3	200.21 (168.64-237.57)	54	0.78 (0.54, 1.13)	0.77 (0.53, 1.13)	51	0.92 (0.63, 1.32)	0.89 (0.61, 1.31)
4	284.20 (237.58-346.90)	60	0.86 (0.60, 1.22)	0.87 (0.59, 1.25)	44	0.91 (0.63, 1.32)	0.72 (0.47, 1.08)
5	452.53 (≥ 346.91)	46	0.69 (0.47, 1.01)	0.73 (0.48, 1.14)	55	0.73 (0.49, 1.07)	0.85 (0.56, 1.28)
P-trend[2]			0.13	0.33		0.31	0.34

1. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

2. Test for trend using median values for each quintile, modeled as a continuous variable.

3. Unadjusted model

4. Adjusted for age, energy, sex, race, region of residence, educational, income, exercise, smoking status, self-report of CAD at baseline, age x race interaction term

5. Sum of total flavonoid I and proanthocyanidin intake

Table S.4.4. Hazard Ratios and 95% CI for incident ischemic stroke by quintile of flavonoid intake in the REGARDS[1] study, adjusted for multiple dietary variables

Quintile	Median Intake (range) mg/day	Multivariable[5]	All dietary variables[6]
Total Flavonoid I[2]			
1	34.31 (≤ 50.86)	1.00 (-)	1.00 (-)
2	66.59 (50.87-83.43)	0.96 (0.74, 1.25)	1.00 (0.76, 1.31)
3	102.97 (83.44-127.05)	0.93 (0.71, 1.22)	0.99 (0.75, 1.32)
4	156.94 (127.06-208.32)	0.73 (0.55, 0.98)	0.79 (0.76, 1.08)
5	296.84 (≥ 208.33)	0.94 (0.71,1.26)	1.02 (0.75, 1.38)
P-trend[3]		0.62	0.89
Anthocyanidin			
1	3.45 (≤ 4.89)	1.00 (-)	1.00 (-)
2	6.29 (4.90-7.86)	0.72 (0.55, 0.95)	0.74 (0.57, 0.97)
3	9.63 (7.87-11.83)	0.72 (0.54, 0.94)	0.76 (0.57, 1.02)
4	14.58 (11.84-18.49)	0.70 (0.53, 0.93)	0.76 (0.56, 1.02)
5	25.28 (≥ 18.50)	0.93 (0.70, 1.23)	1.03 (0.75, 1.41)
P-trend[3]		0.67	0.25
Flavan-3-ol			
1	7.96 (≤ 12.42)	1.00 (-)	1.00 (-)
2	17.75 (12.43-24.00)	0.79 (0.60, 1.10)	0.81 (0.61, 1.07)
3	35.38 (24.01-52.66)	1.05 (0.81, 1.36)	1.09 (0.84, 1.42)
4	87.12 (52.67-119.66)	0.71 (0.54, 0.95)	0.73 (0.55, 0.97)
5	221.00 (≥ 119.66)	0.97 (0.73-1.27)	0.99 (0.75-1.30)
P-trend[3]		0.93	0.86
Flavanone			
1	2.09 (≤ 3.95)	1.00 (-)	1.00 (-)
2	6.34 (3.96-9.98)	0.88 (0.68, 1.16)	0.88 (0.67, 1.15)
3	16.42 (9.99–23.72)	0.82 (0.62, 1.08)	0.83 (0.63, 1.10)
4	34.86 (23.73–47.96)	0.80 (0.61, 1.05)	0.83 (0.61, 1.05)
5	58.10 (≥ 47.97)	0.73 (0.55, 0.95)	0.78 (0.53, 1.14)
P-trend[3]		0.03	0.29
Flavone			
1	0.32 (≤ 0.42)	1.00 (-)	1.00 (-)
2	0.53 (0.43–0.63)	0.88 (0.68, 1.15)	0.90 (0.69, 1.17)
3	0.76 (0.64–0.90)	0.87 (0.66, 1.14)	0.89 (0.67, 1.19)
4	1.12 (0.91-1.45)	1.08 (0.82, 1.42)	1.13 (0.85, 1.52)
5	2.09 (≥ 1.46)	0.94 (0.70, 1.26)	0.99 (0.72, 1.45)
P-trend[3]		0.88	0.53
Flavonol			
1	6.76 (≤ 8.98)	1.00 (-)	1.00 (-)
2	11.28 (8.99-13.53)	1.19 (0.92, 1.55)	1.22 (0.94, 1.58)
3	15.99 (13.54-18.96)	0.89 (0.67, 1.19)	0.92 (0.69, 1.24)
4	22.64 (18.97-27.66)	0.91 (0.68, 1.22)	0.95 (0.70, 1.30)
5	35.75 (≥ 27.67)	1.16 (0.86, 1.57)	1.23 (0.88, 1.71)
P-trend[3]		0.79	0.45

Table S.4.4. Continued

Isoflavone			
1	0.12 (≤ 0.18)	1.00 (-)	1.00 (-)
2	0.25 (0.19-0.33)	1.05 (0.81, 1.37)	1.06 (0.81, 1.37)
3	0.43 (0.34–0.56)	0.97 (0.74, 1.28)	0.98 (0.76, 1.29)
4	0.73 (0.57–1.02)	1.01 (0.76, 1.34)	1.04 (0.77, 1.39)
5	1.68 (≥ 1.03)	1.06 (0.79, 1.44)	1.13 (0.82, 1.56)
P-trend[3]		0.11	0.46
Proanthocyanidin			
1	33.79 (≤ 46.84)	1.00 (-)	1.00 (-)
2	58.73 (46.85-70.91)	0.91 (0.70, 1.20)	0.93 (0.71, 1.23)
3	84.01 (70.92-98.75)	0.92 (0.70, 1.22)	0.96 (0.73, 1.28)
4	116.86 (98.76-141.33)	1.04 (0.78, 1.37)	1.11 (0.83, 1.48)
5	181.48 (≥ 141.33)	0.89 (0.65, 1.23)	0.99 (0.71, 1.40)
P-trend[3]		0.80	0.74
Total Flavonoid II[4]			
1	79.84 (≤ 109.58)	1.00 (-)	1.00 (-)
2	139.34 (109.59-168.63)	0.74 (0.56, 0.97)	0.77 (0.58, 1.02)
3	200.21 (168.64-237.57)	0.83 (0.63, 1.09)	0.88 (0.67, 1.16)
4	284.20 (237.58-346.90)	0.87 (0.66, 1.15)	0.95 (0.71, 1.26)
5	452.53 (≥ 346.91)	0.73 (0.65, 1.01)	0.80 (0.58, 1.10)
P-trend[3]		0.19	0.80

1. Hazard Ratios and 95% confidence intervals estimated using Cox proportional hazards models.

REGARDS, REasons for Geographic and Racial Disparities in Stroke

2. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

3. Test for trend using median values for each quintile, modeled as a continuous variable.

4. Sum of total flavonoid I and proanthocyanidin intakes

5. Adjusted for age, energy, sex, race, region of residence, educational, income, exercise, smoking status, self-report of CAD at baseline, age x race interaction term

6. Multivariable model + potassium, omega 3 fatty acids, whole grain (servings/day), fiber, folate, ß carotene, vitamin C

CHAPTER 5: DIETARY FLAVONOID INTAKE AND INCIDENT CORONARY HEART DISEASE: THE REGARDS STUDY

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ABSTRACT

Background: Flavonoids are dietary polyphenolic compounds with a variety of proposed beneficial cardiovascular effects, but rigorous prospective studies examining the association between flavonoid intake and incident coronary heart disease (CHD) in geographically and racially diverse U.S. samples are limited. The USDA flavonoid databases were recently improved to address missing flavonoid values for cooked foods and adjust for flavonoid losses due to processing, which could lead to measurement error of flavonoid intake.

Objective: Using the new, expanded USDA flavonoid database, we assessed the association between total flavonoid and flavonoid subclass intakes with incident CHD in a biracial and geographically diverse cohort, as well as effect modification by age, sex, race and region of residence.

Methods: Participants were 16,678 black and white, men and women, enrolled in the REGARDS study, a national prospective cohort study, without CHD at baseline, who completed a Block98 food frequency questionnaire (FFQ). Flavonoid intakes were estimated from USDA flavonoid databases. Incident CHD events were participant reported and adjudicated by experts. Quintiles of flavonoid intake were examined as predictors of incident CHD using Cox proportional hazards regression to obtain hazard ratios (HR). Tests for trend used the quintile medians.

Results: Over a mean 6.0 years (±1.9 yrs) of follow-up, 605 CHD events occurred. High flavonoid intake was associated with self-identified white race, exercise, not smoking, education and income. In fully adjusted models, there was an inverse association between anthocyanidin and proanthocyanidin intake and incident CHD (anthocyanidins HR_{Q5vQ1}=0.65 (95% CI 0.50, 0.86), p-trend=0.003; proanthocyanidins HR_{Q5vQ1}=0.62 (95% CI 0.48, 0.81), p-trend=0.002).

There was no association between total flavonoid or other flavonoid subclass intakes and incident CHD.

Conclusions: Reported anthocyanidin and proanthocyanidin intake is inversely associated with incident CHD. There was no significant effect modification by age, sex, race or region of residence.

INTRODUCTION

Black Americans and residents of the Southeastern United States (US), a region also known as the Stroke Belt due to elevated stroke mortality, are at greater risk of coronary heart disease (CHD) mortality and fatal incident CHD than whites and those living elsewhere [28,29,264]. High adherence to a southern dietary pattern, characterized by fried foods, organ meats and sugar-sweetened beverages, is more common among men, non-Hispanic blacks and residents of the Southeastern United States, and has been associated with a 66% increased risk of incident CHD [265]. In contrast, diets high in plant-based foods have been associated with decreased risk of CHD [266,267]. While Americans generally consume inadequate amounts of fruits and vegetables, consumption among black Americans is even less likely to be adequate [268,269], which may be related to socioeconomic or cultural dietary factors that differ between white and black Americans.

Flavonoids are bioactive, polyphenolic compounds found in a wide variety of plant-based foods, including fruits and vegetables, tea, wine, nuts, herbs and spices. Subclasses of flavonoids include anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, isoflavones and proanthocyanidins. Cardioprotective mechanisms for flavonoids have been proposed, including antioxidant and anti-inflammatory action, modulation of lipid metabolism and platelet function and attenuation of hypertension [14]. Consistent with these biological effects, epidemiologic

studies to date suggest a protective effect for flavonoids against CHD mortality [13,16], incident CHD [18,19] as well as other outcomes relevant to CHD, such as arterial stiffness, incident hypertension and type 2 diabetes and improved long-term weight maintenance [21-23,110]. However, no published studies examining flavonoids and incident CHD have enrolled a racially diverse participant population and only one study in the United States has been geographically diverse [13,16]. Furthermore, this field of research has been hampered by a lack of comprehensive food flavonoid composition tables. To address this issue, in 2014 the U.S. Department of Agriculture (USDA) released the Provisional Flavonoid Addendum [25]. Using this new, expanded flavonoid database, we evaluated the association of each of seven flavonoid subclasses and the summed total of flavonoid subclass intakes with incident CHD in a large biracial cohort study. Furthermore, we assessed whether the association between flavonoid intake and CHD differed by age, sex, race and residence in the Southeastern US.

METHODS

Study Participants

The REasons for Geographic and Racial Differences in Stroke (REGARDS) study is a prospective cohort study of 30,239 participants designed to examine regional and racial influences on stroke mortality. English-speaking, community-dwelling, non-Hispanic white and black adults, living in the continental United States and over 45 years old were recruited between 2003 and 2007 using a combination of mail and telephone contacts. Self-reported race and sex were balanced by design, with oversampling from the Southeastern United States, often referred to as the Stroke Belt, including Alabama, Arkansas, Georgia, Louisiana, Mississippi, Tennessee, North Carolina and South Carolina. Within the Stroke Belt, the coastal plains regions of Georgia, North Carolina and South Carolina, often referred to as the Stroke Buckle, experience an even

higher rate of stroke mortality than the rest of the Stroke Belt. The final cohort included 56% residents from the Southeastern U.S. and 44% residents of the contiguous lower 48 states, 42% black and 55% women [174]. Baseline data collection included computer-assisted telephone interview to assess health status and medical history. Trained healthcare professionals conducted in-home examinations using standardized, quality controlled protocols to obtain fasting blood and urine samples, electrocardiograms, blood pressure, height and weight measurements and complete a medication inventory. The Institutional Review Boards of all participating universities approved this study and written informed consent was obtained from all participants.

Dietary Assessment

A Block98 Food Frequency Questionnaire was left with all study participants at the conclusion of the in-home visit and returned by mail. This self-administered FFQ is a 107-item questionnaire developed by NutritionQuest (Berkeley, CA) and has been validated in populations relevant to REGARDS [213,214,270]. The Block98 FFQ inquires about plant-based foods with high flavonoid content, including fruits, vegetables, tea, nuts and wine. NutritionQuest provided mean daily intake estimates, in grams, for FFQ items. Those who completed < 85% of the FFQ and with reported caloric intake per day of < 800 kcal or > 4200 kcal for men and < 500 kcal or > 3500 kcal for women were excluded from analyses.

Estimation of Flavonoid Intake

Specific flavonoid intakes of interest were anthocyanidins, flavan-3-ols, flavanones, flavone, flavonols, isoflavones, and proanthocyanidins, which are oligomers and polymers of flavan-3-ols, as well as total flavonoid intake. The USDA Database for the Proanthocyanidin Content of Selected Foods and the USDA Provisional Flavonoid Addendum to the USDA Food and Nutrient Database for Dietary Studies (FNDDS), 4.1, provide flavonoid content information for

complementary sets of flavonoids, which are presented as aglycone equivalents [25,46]. The flavonoids included in these databases and used to estimate flavonoid intakes in this study are summarized in **table S.5.1**. The Provisional Flavonoid Addendum is the most recent comprehensive food flavonoid database, providing data for 29 flavonoids in six subclasses for 7,147 foods and beverages in the FNDDS, version 4.1 (2007-2008) and accounts for the effects of processing and cooking on flavonoids better than previous releases of the USDA flavonoid databases [25]. Foods/beverages, portions, and nutrient descriptions are updated in the FNDDS every two-years based on 24-hour recalls in the dietary intake component of the nationally representative National Health and Human Nutrition Examination Survey (NHANES). In contrast, the USDA Database for the Proanthocyanidin Content of Selected Foods was released over ten years ago, and has not been updated [46]. The Provisional Flavonoid Addendum and Proanthocyanidin Database are largely complementary; though contain overlapping information about flavan-3-ols, which are identified as monomers in the Proanthocyanidin Database. Given the methodological differences between the databases, when a food was included in both databases, and if a flavan-3-ol value (Addendum), and monomer value (Proanthocyanidin Database) were both available, only the current flavan-3-ol value from the Provisional Flavonoid Addendum was used. Furthermore, two different measures of total flavonoid intake were used. One included only flavonoid values obtained from the Provisional Flavonoid Addendum (total I) and a second one added proanthocyanidin intake (total II).

Food items comprising the Block98 FFQ were matched to corresponding food items in the Provisional Flavonoid Addendum, using the unique 8-digit FNDDS food code, and the 5-digit USDA Standard Reference food codes in the Proanthocyanidin Database. When necessary, the USDA FNDDS-SR links file was used as a crosswalk between datasets. For food items that

included only one food (eg, bananas), the flavonoid values for the matching food in the USDA databases was used. Calculations for combined items on the FFQ, such as "apples or pears," were weighted averages using NHANES-based per capita consumption, consistent with weights used in the Block98 FFQ. Items on the FFQ with multiple ingredients (eg, pizza) were assigned flavonoid values consistent with the USDA standard recipes. USDA recipes did not differentiate between foods eaten at home or outside of the home. Flavonoid values provided in the Provisional Flavonoid Addendum already account for processing factors. Estimated daily flavonoid intake for each participant was calculated by multiplying the reported amount (grams) of food consumed by the flavonoid content of the corresponding food (mg flavonoid/100 grams of food) and summed across foods.

Acute CHD Events

Participants were contacted every six months with active telephone surveillance. Hospitalizations reported by living participants or proxies triggered medical record retrieval. Deaths were detected by report of next-of-kin, through online sources (eg. Social Security Death Index) or the National Death Index. Proxies or next-of-kin were interviewed about the circumstances surrounding death including the presence of chest pain. Cause of death and cardiovascular outcomes were adjudicated using medical records, death certificates and autopsy reports following published guidelines and have been described previously [28]. Only definite or probable myocardial infarctions (MI) and CHD deaths occurring through December 31, 2011 were included in this analysis.

Statistical Analyses

We created quintiles of reported total and subclass flavonoid intakes defined from the total study population because gender-specific intakes were not meaningfully different. Median

intake and ranges of intakes for total flavonoids and each flavonoid subclass were calculated. Flavonoid values were energy-adjusted using the residual method [271]. Baseline characteristics were summarized by quintiles of total flavonoid I intake. The association between quintiles of flavonoid intake and the risk of incident CHD events was examined using Cox proportional hazards models. Participants were censored at the date of CHD event, loss to follow-up or last CHD adjudication, whichever occurred first. After verifying the proportional hazards assumption, models were built by sequential adjustment for potential confounders by first adding age, energy intake and sex, followed by other demographic factors (race and region of residence), socioeconomic factors (household income and educational attainment), and health behaviors (smoking status, pack-years smoking and physical activity). These variables have been previously described [237]. Variables were not included in the final model if their inclusions did not change the HR in the top quintile of exposures more than 10%. The most parsimonious model without dietary variables included age, energy intake, sex, as well as self-identified race, region of residence, socioeconomic, and health behavioral factors. Next, we considered the following dietary variables as potential confounders: beer and liquor intake, supplement intake, whole grains, fatty acids, fiber, folate, vitamin C, vitamin E, β-carotene, potassium, magnesium, sodium and percent of calories from sweets (sweetened cereals and beverages, jelly, and sugar/honey added to coffee or tea). Because dietary variables can be strongly intercorrelated, they were added to the model individually and retained if they were independently associated with incident CHD. Percent of calories consumed from sweets was the only dietary variable independently associated with incident CHD. Wine intake was not included in models to avoid over-adjusting for certain sources of flavonoids. Because cardiovascular risk factors and related medication use were likely mediators along the causal pathway between flavonoid intake and

incident CHD, we conducted exploratory analyses, adding terms to the model for BMI, history or use of medications for hyperlipidema, diabetes, and hypertension, as well as aspirin use. The most parsimonious model included age, energy intake, sex, self-identified race, region of residence, socioeconomic, and health behavior factors and percent calories from sweets. We conducted stratified analyses based on *a priori* (age at baseline, race, sex and region of residence) and *post hoc*- identified (education, physical activity, smoking status) potential effect modifiers. Interaction terms between quintiles of flavonoid intake and these variables were also added to models and likelihood ratio chi-square tests were used to formally test for statistical interaction. Trend tests were conducted by assigning each quintile its median value and modeling the exposure as a continuous variable. Sensitivity analysis using multiply-imputed missing covariates was conducted to assess possible bias due to exclusion of participants with missing information on three key covariates, including smoking status (n=66), education (n=8), or physical activity (n=233).

We also examined the association between selected FFQ food items and coronary heart disease endpoints using multivariable models similar to those used for flavonoid intake. We included foods if they comprised at least 1% of reported total flavonoid intake. These foods included tea (63%), apples or pears (8%), cakes (6%), citrus fruits/juices (5%), canned fruit (4%), wine (4%), 100% fruit juice excluding orange (3%), peanuts/nuts (2%), legumes (2%), and berries (1%). Foods were categorized into quintiles, except for wine and tea. Wine was a composite of primarily red wine, white wine and wine cocktails weighted by NHANES-based population intake. Wine consumption was categorized as nondrinkers, ≤1 drink/wk, 2-4 drinks/wk, and ≥5 drinks/wk. Because neither the FFQ nor the Provisional Flavonoid Addendum differentiate the type of tea consumed, values are a composite of 84% black tea and 16% green

tea based on market-share data and intake was categorized as nondrinkers, 1-3 cups/month, 1-6 cups/wk and ≥1 cup daily. Final models in food analyses were adjusted for age, caloric intake, sex, race, region of residence, income, education, physical activity, smoking status and percent of calories from sweets.

To investigate the effect of energy misreporting, flavonoid analyses were repeated after energy misreporters were excluded using the Goldberg cutoffs [248,249]. Basal metabolic rate was estimated using the Schofield equations for height, weight and age [250] and physical activity level was assumed to be 1.55. Analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

There were 21,096 participants (70%) who returned a FFQ with complete dietary data, defined by completing at least 85% of the FFQ items and plausible reported caloric intake. After excluding participants with a history of CHD at baseline (n=3,946), missing information on outcome (n=175) or key covariates (n=297), 16,678 participants were available for this analysis. During a mean follow-up of 6.0 ± 1.9 years, 589 incident CHD events were observed, 221 in women, and 368 in men. The participant flow diagram is in **figure 5.1**. Those excluded from the dietary subsample were more often black (61% versus 39%), less educated (20% versus 10% with less than a complete high school education) and had a lower reported household income (24% versus 16% <$20,000/year), when compared to those in the dietary subsample. There was no difference in the proportion of incident CHD events experienced in the dietary subsample as compared to those in the full sample.

Mean and median total flavonoid intakes for women were 234 mg and 131 mg, respectively, and for men they were 227 mg and 131 mg, respectively. Mean and median reported daily caloric intakes for women were 1561 kcal and 1470 kcal, respectively and for men

were 1871 kcal and 1758 kcal, respectively. The top food sources of flavonoids are listed in **table 5.1**. Baseline characteristics by quintiles of flavonoid I intake are summarized in **table 5.2**. The distribution of baseline characteristics did not differ when total flavonoid intake II was used instead (flavonoid intake I plus proanthocyanidins). Individuals with higher total flavonoid intake were more likely to be white, more educated, and less sedentary, to have higher reported household income and never to have smoked.

The associations of quintiles of total flavonoid and flavonoid subclasses with incident CHD in the total cohort are shown in **table 5.3**. There was a statistically significant inverse association, with a linear trend, between anthocyanidin intake and proanthocyanidin intake and incident CHD in models I and II with 35% and 38% relative risk reduction after multivariable adjustment, respectively. Models including cardiovascular risk factors yielded minimally attenuated effect estimates with widened confidence intervals (anthocyanidins HR_{Q5vQ1}: 0.70, 95% CI: 0.53, 0.93, p-trend: 0.03; proanthocyanidins HR_{Q5vQ1}: 0.65, 95% CI: 0.50, 0.85, p-trend: 0.01 and results for remaining flavonoid subclasses are in **table S.5.2**. The statistically significant associations, with a significant linear trend, between flavones and isoflavones and incident CHD seen in model I were attenuated in model II. Because reported isoflavone intake in the cohort was low we also compared quintiles 1-4 combined to quintile 5, and extreme deciles of intake, though results remained null (data not shown). Similarly, the statistically significant linear trend for the association between flavonols and incident CHD in model I was no longer statistically significant after full covariate adjustment. There was no statistically significant association between flavan-3-ol, flavanone, or total flavonoid intake and CHD. When energy misreporters were excluded in sensitivity analyses, estimates were attenuated for anthocyanidins

(HR_{Q5vQ1}: 0.70, 95% CI: 0.50, 0.96) and strengthened for proanthocyanidins (HR_{Q5vQ1}: 0.56, 95% CI: 0.41, 0.80), suggesting some bias due to misreporting (**table S.5.2**).

Analyses stratified by the *a priori* factors, age at baseline, sex, race, and region of residence or post-hoc factors, education, physical activity and smoking status, did not reveal differences in the association between flavonoid intake and incident CHD, nor were there statistically significant interactions (all p>0.10). Analyses using multiple imputation for missing covariates yielded similar results. Stratified results by sex and race are available in the online supplement (**Tables S.5.3 and S.5.4**).

In food-based analyses, there was a statistically significant association between the consumption of several foods sources of flavonoids, including apples or pears, berries, and wine, and incident CHD. There was an inverse association between consuming approximately ≥ 3 small apples or pears per week or ≥ 2 servings of berries per week and incident CHD (apples HR_{Q5vQ1}: 0.75, 95% CI: 0.57, 0.99, p-trend: 0.02; berries HR_{Q5vQ1}: 0.69, 95% CI: 0.52, 0.92, p-trend: 0.01). There was a graded inverse association between wine consumption and incident CHD compared to nondrinkers (≤1 drink/wk: HR=0.74, 95% CI: 0.61, 0.91; 2-4 drinks/wk: HR=0.68, 95% CI: 0.49, 0.97; ≥5 drinks/wk: HR=0.54, 95% CI: 0.38, 0.75). There was no statistically significant association between incident CHD and any other main foods contributing to total flavonoid intake.

DISCUSSION

This is the first prospective cohort study to evaluate the association of total flavonoid and flavonoid subclass intakes, with incident CHD in a biracial cohort using the newly expanded USDA Provisional Flavonoid Addendum. We were able to show that subclasses of flavonoids are more relevant to incident CHD than total flavonoid intake. Greater intakes of anthocyanidins

and proanthocyanidins were associated with 35% and 38% reduced relative risk of incident CHD, respectively, after adjustment for potential confounders. The statistically significant inverse association between proanthocyanidin intake and incident CHD was a novel finding. Our findings differ from recent results from a study of flavonoid intake and incident CVD in the Framingham Offspring Cohort, which found no association between total flavonoid or flavonoid subclass intakes and incident CHD [19]. However, the Framingham Offspring Cohort may be particularly attuned to modifiable cardiovascular risk factors. The only other previous study evaluating proanthocyanidin or anthocyanidin intake and incident CHD was conducted in the Nurses' Health Study II [18]. In NHS II, anthocyanidin intake was associated with a 32% reduction of relative risk of incident CHD, which was similar to the 35% reduction seen in this study. Anthocyanidin intake was comparable between this study and NHS II. Conversely, participants in our study who reported the highest quintile of proanthocyanidin intake experienced a 38% relative risk reduction as compared to only 17% risk reduction in incident CHD in NHS II. In addition to using a different version of the USDA flavonoid databases and a different FFQ, the NHS II enrolled female healthcare providers between 25-42 years old, which is a younger and more health conscious cohort than the REGARDS study, which may explain differences in the effect estimates.

There was no statistically significant effect modification by a variety of factors including age, sex, race, region of residence, educational attainment, smoking or physical activity. Black participants reported lower flavonoid intakes than their white counterparts. This may be due to lower consumption of plant-based foods in this group, consistent with previous studies showing that black participants in REGARDS more often consumed a Southern-style dietary pattern, which is associated with incident CHD [265].

Observational and experimental evidence supports the inverse association of dietary anthocyanidin with incident CHD and CHD risk factors. In addition to a lower risk of incident CHD, high anthocyanidin intake may prevent onset of type 2 diabetes [104]. Most recently, physiological levels of anthocyanidin metabolites were found to significantly reduce interleukin 6 and vascular cell adhesion molecule-1 in human vascular endothelial cells stimulated with oxidized LDL cholesterol or cluster of differentiation 40 ligand, suggesting that anthocyanidin metabolites may modulate expression of important inflammatory mediators [272]. The association we found for dietary proanthocyanidins is particularly intriguing, as compounds found in the proanthocyanidin subclass are not well understood. Proanthocyanidins were not associated with CHD mortality in previous epidemiologic studies and human intervention studies are scarce [16,17]. In these previous studies, outcomes were defined by ICD codes for ischemic heart disease (410-414), including subacute MI, old MI, angina pectoris and other chronic ischemic heart disease, which is less specific than expert-adjudicated incident acute MI and CHD death. Animal and *in vitro* studies suggest that proanthocyanidins may have a role in reducing adiposity, a risk factor for CHD, through inhibition of digestive enzymes in the small intestine, modulation of neuropeptides involved in satiety and influence on lipid metabolism [273]. Proanthocyanidins also appear to have anti-thrombotic and antihypertensive effects in animal models [273,274].

Food-based analyses support the inverse associations between anthocyanidins, proanthocyanidins, and incident CHD. Apples and pears, with peels, are rich sources of proanthocyanidins and other flavonoids in smaller amounts. Berries are rich sources of anthocyanidins and intakes ≥3 servings/week are associated with a 34% reduction in relative risk of incident CHD [18]. Wines are notable sources of polyphenols, though red wines are particularly rich in flavonoids, including anthocyanidins, proanthocyanidins, and other subclasses in smaller

quantities. Though wine, apples, and berries are characteristically high in specific flavonoid subclasses, the effect of each subclass cannot be disentangled in food-based analyses. These foods may represent naturally potent mixtures of flavonoids.

Strengths of this study include the large biracial, geographically diverse sample, prospective design and excellent ascertainment of CHD outcomes. The flavonoid database used is the most comprehensive database available to assess flavonoid intake in the U.S. population. The racial and geographic diversity of the study population contribute to the generalizability of the results. Some limitations should be mentioned. Assessment of dietary flavonoids is susceptible to measurement error, as FFQs cannot capture all potential dietary sources of flavonoids. The Block98 FFQ was not specifically designed to measure flavonoid intake, leading to potential measurement error. However, the use of a detailed, comprehensive food composition table and detailed FFQ, should mitigate measurement error by capturing most flavonoid intake for Americans, especially since the exposure was rank ordered, rather than absolute intake. Diet was only assessed at baseline; therefore changes in dietary composition could not be assessed. Though there was a ten-year interval between the release of the Block98 FFQ and then end of enrollment in REGARDS in 2007, analysis of NHANES data over a similar time period indicate that flavonoid intake and the distribution of dietary flavonoid sources remained stable [34], therefore the Block98 was likely able to capture flavonoid intake accurately. While the flavonoid content of plants varies depending on cultivars, growing conditions and storage, values used in this study were mean flavonoid values for foods, which are the most appropriate measure to use in a large epidemiologic study, in which participants likely consumed various foods from various sources. Additionally, while not all REGARDS participants returned the dietary assessment, and some covariates were missing, there was no material difference in

results when analyses were repeated after imputation of missing covariates. Energy misreporting may lead to bias in food surveys research, including those utilizing FFQs. In this study, sensitivity analyses excluding energy under/over-reporters yielded estimates that were not meaningfully different from the main analysis. As in all observational studies, confounding by unmeasured factors is possible. Residual confounding by smoking status is possible because features beyond smoking status influence CVD risk prediction. While time since quitting and age at quitting were not measured in this study, inclusion of pack-years in models did not change estimates. Residual confounding by other dietary factors is unlikely, as many potential dietary confounders were evaluated and were not independently associated with incident CHD, except caloric intake from sweetened foods. Finally, statistical significance should be interpreted cautiously, as some significant findings may be due to chance, given the number of comparisons in this study.

Conclusion

We found that higher reported intake of anthocyanidins and proanthocyanidins was associated with a lower risk of incident CHD after adjusting for demographic, socioeconomic, health behavior and dietary factors. Similarly, higher reported intake of apples/pears, berries and wine, were also inversely associated with incident CHD. There was no effect modification by age, sex, race or region of residence, though non-Hispanic blacks reported lower flavonoid intake, which may contribute to a greater CHD burden. Higher consumption of foods rich in anthocyanidins and proanthocyanidins may explain some of the cardioprotective effects of plant-rich diets and their ability to prevent CHD should be evaluated in clinical trials.

Table 5.1. Proportional contribution of Block98 FFQ items to total flavonoid and flavonoid subclass intake for 16,678 participants without coronary heart disease at baseline in the REasons for Geographic and Racial Differences in Stroke (REGARDS) Study, 2003-2007.

Total Flavonoid I[1]		Total Flavonoid II[2]	
Food source	%	Food source	%
Tea[3]	79	Tea[3]	63
Orange juice	9	Apple or pears	8
Orange, raw	3	Cake[7]	6
Wine[4]	2	Orange juice	4
100% real fruit juice, not orange	2	Pies and cobbler[8]	4
Apples or pears, raw	2	Canned fruit[6]	4
Other vegetables[5]	2	Wine[4]	4
Salad greens	<1	100% real fruit juice, not orange	2
Canned fruit[6]	<1	Peanuts and nuts	1
Grapefruit, raw	<1	Beans	1
Anthocyanidins		**Flavan-3-ols**	
Food source	%	Food source	%
Wine[4]	27	Tea[3]	94
100% real fruit juice, not orange	16	Apples or pears	1
Berries	14	100% real fruit juice, not orange	1
Cole slaw	8	Bananas	1
Vegetable stews[9]	8	Wine[4]	1
Yogurt with fruit	7	Canned fruit[6]	1
Other fruits[8]	6		
Canned fruit[6]	4		
Other vegetables[5]	4		
Apples or pears	3		
Flavanone		**Flavone**	
Food source	%	Food source	%
Orange juice	67	Other vegetables[5]	53
Oranges	21	Salad greens	14
Grapefruit	7	Tea[3]	7
100% real fruit juice, not orange	2	Spinach	5
Liquor/cocktails	1	Cantaloupe	4
Tomato	<1	Orange	3
		Vegetable stews[9]	3
		Apple or pear	2
		Broccoli	2
		Grapefruit	1

Flavonol		Isoflavones	
Food source	%	Food source	%
Tea[3]	44	Soymilk	48
Salad greens	18	Peanuts and nuts	18
Other vegetables[5]	12	Tofu	17
Dark leafy greens	8	Other vegetables[5]	6
Apple or pears	6	Meat substitutes	3
Broccoli	4	Protein bars	2
100% real fruit juice, not orange	3	Bean soups	2
Wine[4]	3	Protein shakes	1
Proanthocyanidins			
Food source	%		
Apples or pears	18		
Cake[7]	16		
Pies and cobbler[8]	10		
Tea[3]	9		
Canned fruit[6]	8		
Beans	6		
Peanuts and nuts	6		
Wine[4]	6		
100% real fruit juice, not orange	4		
Berries	3		

1. Sum of flavonoid subclasses in the USDA Provisional Flavonoid Addendum (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, isoflavones)

2. Sum total flavonoid I and proanthocyanidins

3. In the Provisional Flavonoid Addendum, flavonoid values for tea are a composite consisting of 84% black tea and 16% green tea, based on published market share data.

4. A composite of red wine, white wine, cooking wine and sangria, weighted by National Health and Nutrition Examination Survey (NHANES) population-based reported intakes

5. Includes pumpkin, squash, peppers, onion, artichokes, asparagus, lima beans, bean sprouts, beets, brussel sprouts, cauliflower, celery, eggplant, mushrooms, okra, and parsnips that are cooked from fresh or frozen. Anthocyanidins primarily from eggplant; Flavones from pumpkins, squash, peppers, celery, okra; Flavonols from asparagus, peppers, brussels sprouts, okra, onion; Isoflavones from soybeans, bean sprouts

6. Canned fruit includes apricots, cherries, plums, peaches, pears, fruit cocktail and applesauce
7. Includes fruit, chocolate, and spice cakes; important sources of flavonoids include fruits, chocolate, and cinnamon.
8. Includes fruit cobblers, chocolate pies, and turnovers; important sources of flavonoids include fruits, chocolate and cinnamon
9. Mix of vegetables, with or without meat, prepared as a stew, excludes soups. Anthocyanidins primarily from eggplant; Flavones primarily from squashes, peppers, herbs
10. Anthocyanidins primarily from cherries, plums, red grapes, fruit salads

Table 5.2. Baseline characteristics for 16,678 participants without CHD at baseline in the REasons for Geographic and Racial Differences in Stroke (REGARDS) Study 2003-2007 by Quintile of Total Flavonoid Intake (total I)[1]

	Quintiles of Total Flavonoid I Intake					
	Q1 n=3305	**Q2** n=3341	**Q3** n=3362	**Q4** n=3362	**Q5** n=3321	**p-value**[2]
Age, y	63.4 (9.0)[3]	64.4 (9.0)	64.7 (9.2)	64.6 (9.2)	64.2 (8.9)	0.002
Energy intake, kcal	2039 (548)	1473 (530)	1511 (588)	1653 (611)	1774 (779)	<0.001
BMI, kg/m^2	29.4 (6.3)	29.0 (6.2)	28.8 (5.9)	29.1 (6.0)	28.7 (6.0)	0.003
Male, %	1761 (53.3)	1297 (38.8)	1233 (36.8)	1317 (39.2)	1272 (38.3)	<0.001
White, %	2070 (62.6)	2011 (60.2)	2034 (60.7)	2241 (66.7)	2643 (79.6)	<0.001
Region[4], %						<0.001
Stroke Belt	1146 (34.7)	1080 (32.3)	1099 (32.8)	1174 (34.9)	1227 (37.0)	
Stroke Buckle	575 (19.2)	659 (20.0)	681 (19.8)	818 (23.2)	909 (27.3)	
Not Stroke Belt	1584 (47.9)	1602 (47.8)	1569 (46.9)	1370 (40.8)	1185 (35.7)	
Physical activity, %						<0.001
None	1174 (35.5)	1113 (33.3)	954 (28.5)	1019 (30.3)	1028 (30.9)	
1-3 times/wk	1174 (35.5)	1254 (37.5)	1359 (40.6)	1294 (38.5)	1238 (37.3)	
≥4 times/wk	957 (25.9)	974 (29.2)	1036 (30.9)	1049 (31.2)	1055 (31.8)	
Smoking status, %						<0.001
Never	1297 (39.2)	1593 (46.1)	1654 (49.4)	1667 (49.6)	1702 (51.3)	
Past	1356 (41.0)	1365 (40.9)	1334 (39.8)	1331 (39.6)	1229 (37.0)	
Current	364 (11.0)	286 (8.6)	269 (8.0)	267 (7.9)	233 (7.0)	
Education, %						<0.001
<HS	364 (11.0)	286 (8.6)	269 (8.0)	267 (7.9)	233 (7.0)	
HS grad	950 (28.4)	806 (24.1)	735 (22.0)	794 (23.6)	862 (25.9)	
Some college	901 (27.6)	900 (26.9)	942 (28.1)	920 (27.4)	949 (28.6)	
College grad	1090 (32.8)	1349 (40.4)	1403 (41.9)	1381 (41.0)	1277 (38.5)	
Income, %						0.001
Refused	367 (11.1)	406 (12.2)	381 (11.4)	382 (11.4)	386 (11.6)	

<$20,000	568 (17.2)	487 (14.5)	450 (13.4)	497 (14.8)	441 (13.3)	
$20,000-$34,000	788 (23.4)	765 (22.9)	763 (22.8)	831 (24.7)	786 (23.7)	
$35,000-$74,000	1036 (31.4)	1067 (31.9)	1102 (32.9)	1047 (31.1)	1070 (32.2)	
≥$75,000	546 (16.5)	616 (18.4)	653 (19.5)	605 (18.0)	638 (19.2)	
Alcoholic drinks/wk	2.9 (8.9)	2.6 (7.3)	2.4 (5.4)	2.2 (5.4)	1.9 (6.0)	<0.001
Dietary fiber (g/d)	16.5 (8.4)	14.3 (7.5)	15.5 (7.9)	16.2 (8.2)	16.9 (8.4)	<0.001
Saturated fat (g/d)	27.0 (9.5)	17.6 (7.5)	17.2 (8.5)	19.2 (9.1)	21.0 (10.2)	<0.001
Polyunsaturated fat (g/d)	23.5 (10.5)	16.0 (7.8)	15.7 (8.5)	17.7 (9.2)	19.3 (10.1)	<0.001
Omega-3 fats (g/d)	1.9 (0.7)	1.4 (0.7)	1.4 (0.7)	1.5 (0.8)	1.7 (0.9)	<0.001
Vitamin C without supplements (mg/d)	84.6 (55.0)	92.2 (55.1)	118.9 (64.3)	121.0 (78.8)	115.6 (77.3)	<0.001
Vitamin E (a-TE/d)	11.0 (5.2)	8.5 (4.3)	8.7 (4.5)	9.5 (4.8)	10.0 (4.9)	<0.001
Beta-carotene (µg/d)	3323 (2797)	3152 (2456)	3576 (2892)	3687 (3119)	3777 (3177)	<0.001
Folate without supplements (µg/d)	352 (153)	297 (135)	322 (143)	345 (146)	373 (153)	<0.001
% kcal from sweets	17.6 (11.2)	14.3 (9.6)	13.0 (8.7)	14.5 (9.2)	16.1 (10.0)	0.86
Whole grains (srv/d)	1.4 (1.3)	1.5 (1.3)	1.5 (1.2)	1.6 (1.3)	1.6 (1.4)	<0.001
Sodium (mg/d)	2767 (1036)	1987 (813)	1977 (885)	2165 (919)	2337 (988)	<0.001
Potassium (mg/d)	2697 (1034)	2294 (938)	2536 (963)	2681 (1016)	2889 (1068)	<0.001

[1] Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes.

[2] Data were analyzed using ANOVA and chi-square tests for continuous and categorical variables, respectively.

[3] Mean (SD) (all such variables)

[4] Stroke Belt includes Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee ;Stroke Buckle includes coastal plains of Georgia, North Carolina and South Carolina, Non-Belt includes remaining area in lower 48 contiguous states. Regions are mutually exclusive.

[5] Answer to "How many times per week do you engage in intense physical activity, enough to work up a sweat?"

Table 5.3. Hazard ratios (HRs) and 95% confidence interval (CI) for incident coronary heart disease by quintile of energy-adjusted flavonoid intake for 16,678 participants in the REGARDS study[1]

	Q1	Q2	Q3	Q4	Q5	*P – trend*[3]
Total flavonoid I, mg/d[2]	46 (≤ 72)	92 (73-113)	141 (114-182)	251 (183-352)	599 (≥ 353)	
Model I[4]	1.00	0.94 (0.72, 1.21)	0.77 (0.59, 1.00)	0.80 (0.62, 1.04)	1.02 (0.80, 1.30)	*0.27*
Model II[5]	1.00	1.01 (0.78, 1.31)	0.86 (0.65, 1.12)	0.89 (0.68, 1.15)	1.13 (0.88, 1.45)	*0.32*
Anthocyanidins, mg/d	4.0 (≤ 5.8)	7.3 (5.9-8.7)	10.3 (8.8-12.2)	14.8 (12.3-18.5)	24.7 (≥ 18.6)	
Model I	1.00	0.78 (0.62, 0.99)	0.74 (0.62, 0.95)	0.62 (0.48, 0.80)	0.53 (0.41, 0.69)	*<0.001*
Model II	1.00	0.82 (0.64, 1.05)	0.83 (0.65, 1.07)	0.72 (0.55, 0.94)	0.65 (0.50, 0.86)	*0.003*
Flavan-3-ols, mg/d	8 (≤ 25)	38 (26-50)	68 (51-112)	187 (113-278)	529 (≥ 279)	
Model I	1.00	0.87 (0.66, 1.15)	1.01 (0.77, 1.32)	0.88 (0.68, 1.15)	1.09 (0.86, 1.41)	*0.22*
Model II	1.00	0.89 (0.68, 1.18)	1.06 (0.81, 1.39)	0.92 (0.71, 1.20)	1.15 (0.89, 1.48)	*0.14*
Flavanones, mg/d	2 (≤ 10)	9 (10–14)	17 (15–19)	34 (20–46)	58 (≥ 46)	
Model I	1.00	0.94 (0.72, 1.23)	0.77 (0.59, 1.02)	0.96 (0.75, 1.24)	0.81 (0.63, 1.04)	*0.22*
Model II	1.00	0.96 (0.74, 1.23)	0.83 (0.63, 1.09)	1.07 (0.83, 1.37)	0.91 (0.70, 1.18)	*0.82*
Flavones, mg/d	0.4 (≤ 0.51)	0.60 (0.52–0.69)	0.80 (0.70–0.94)	1.10 (0.95-1.45)	2.09 (≥ 1.46)	
Model I	1.00	0.88 (0.68, 1.12)	1.04 (0.81, 1.32)	0.89 (0.69, 1.14)	0.75 (0.58, 0.99)	*0.01*
Model II	1.00	0.96 (0.75, 1.24)	1.19 (0.93, 1.53)	1.08 (0.83, 1.41)	0.93 (0.70, 1.23)	*0.54*
Flavonols, mg/d	7 (≤ 9)	11 (9-14)	16 (14-19)	23 (19–28)	36 (≥ 28)	
Model I	1.00	0.84 (0.66, 1.07)	0.77 (0.59, 0.99)	0.79 (0.61, 1.01)	0.83 (0.64, 1.06)	*0.03*
Model II	1.00	0.90 (0.71, 1.15)	0.86 (0.66, 1.12)	0.90 (0.70, 1.16)	0.97 (0.75, 1.26)	*0.97*
Isoflavones, mg/d	0.1 (≤ 0.27)	0.39 (0.28-0.49)	0.57 (0.50–0.67)	0.79 (0.68–0.99)	1.55 (≥ 1.00)	
Model I	1.00	0.68 (0.53, 0.88)	0.66 (0.50, 0.88)	0.81 (0.62, 1.06)	0.65 (0.50, 0.84)	*0.01*
Model II	1.00	0.72 (0.56, 0.94)	0.72 (0.55, 0.96)	0.92 (0.70, 1.20)	0.77 (0.62, 1.01)	*0.35*
Proanthocyanidins, mg/d	42 (≤ 59)	70 (60-81)	91 (82-103)	117 (104-136)	168 (≥ 137)	
Model I	1.00	0.68 (0.53, 0.87)	0.58 (0.53, 0.76)	0.68 (0.53, 0.86)	0.51 (0.40, 0.67)	*<0.001*
Model II	1.00	0.73 (0.57, 0.93)	0.67 (0.51, 0.86)	0.80 (0.63, 1.03)	0.62 (0.48, 0.81)	*0.002*
Total Flavonoids II, mg/d[#]	108 (≤ 149)	180 (150-212)	250 (213-298)	367 (299-478)	717 (≥ 479)	
Model I	1.00	0.87 (0.68, 1.11)	0.71 (0.54, 0.92)	0.77 (0.60, 1.00)	0.87 (0.68, 1.11)	*0.47*
Model II	1.00	0.94 (0.73, 1.20)	0.80 (0.61, 1.04)	0.89 (0.69, 1.14)	0.97 (0.76, 1.25)	*0.20*

1. REasons for Geographic and Racial Disparities in Stroke study (REGARDS). Hazard ratios (95% CI) were calculated with the use of Cox proportional hazards models.

2. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

3. P-trend values were calculated with the use of the Wald test.

4. Model I: adjusted for age, energy and sex

5. Model II: model adjusted for age, energy, sex, race, region of residence, educational attainment, household income, exercise, smoking status, percent calories from sweets

6. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, isoflavone and proanthocyanidin intake.

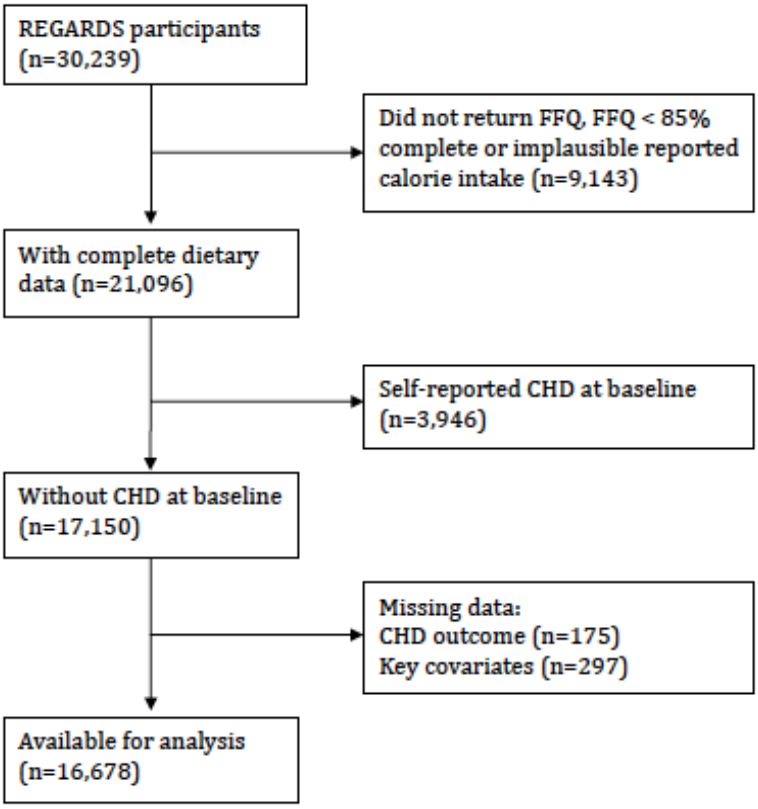

Figure 5.1. Participant flow diagram to determine analytic cohort of 16,678 REGARDS participants without self-reported coronary heart disease at baseline.

Table S.5.1. Flavonoid subclasses, compounds and common food sources included in the US Department of Agriculture flavonoid databases

USDA Database	Flavonoid subclass (common food sources)	Flavonoid Compounds
Provisional Flavonoid Addendum[25]	Anthocyanidins (blueberries, strawberries, red wine)	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin
	Flavan_3_ols (apples, tea, chocolate)	Catechin, Epicatechin, Epicatechin 3-gallate, Epigallocatechin, Epigallocatechin 3-gallate, Gallocatechin, Theaflavin, Theaflavin 3-gallate, Theaflavin 3'-gallate, Theaflavin 3,3'-digallate, Thearubigins
	Flavanones (citrus fruit and juices)	Eriodictyol, Hesperetin, Naringenin
	Flavones (celery, parsley, green pepper)	Apigenin, Luteolin
	Flavonols (onion, apple, broccoli)	Isorhamnetin, Kaempferol, Myricetin, Quercetin
	Isoflavones (tofu, soymilk, soy-based foods)	Daidzein, Genistein, Glycitein
Proanthocyanidin[46]	Proanthocyanidins (apples, chocolate, red wine)	Monomers, dimers, trimers, 4-6mers, 7-10mers, polymers

1. Sebastian RS, Goldman JD, Martin CL, Steinfeldt LC, Enns CW, Moshfegh AJ. Flavonoid Values for USDA Survey Foods and Beverages 2007-2008: Provisional Flavonoid Addendum to the USDA Food and Nutrient Database for Dietary Studies,4.1, and Flavonoid Intake Files from What We Eat in America (WWEIA), National Health and Nutrition Examination Survey (NHANES) 2007-2008. In: U.S. Department of Agriculture ARS, Food Surveys Research Group., ed. Beltsville, MD, 2014.

2. U.S. Department of Agriculture Agricultural Research Service. USDA database for the proanthocyanidin content of selected foods. Beltsville, MD: Agricultural Research Service Nutrient Data Laboratory, 2004.

Table S.5.2. Hazard ratios (HR) and 95% confidence intervals (CI) for incident coronary heart disease, comparing extreme quintiles of total flavonoid and flavonoid subclass intakes, using two different criteria for energy reporting, in the REGARDS study[1]

	Model 1[6]	Model 2[7]	Model 3[8]	Model 4[9]	Model 5[10]	Model 6[11]
Flavonoid subclass (mg/d)						
Total Flavonoid I[2]						
Sex-specific[3]	1.02 (0.80, 1.30)	1.13 (0.88, 1.45)	1.13 (0.88, 1.45)	1.22 (0.94, 1.58)	1.07 (0.83, 1.38)	1.14 (0.87, 1.48)
Goldberg[4]	0.98 (0.74, 1.31)	1.06 (0.80, 1.42)	1.06 (0.80, 1.42)	1.16 (0.86, 1.56)	0.98 (0.73, 1.32)	1.05 (0.77, 1.44)
Anthocyanidin						
Sex-specific	0.53 (0.41, 0.69)	0.70 (0.53, 0.92)	0.65 (0.50, 0.86)	0.70 (0.51, 0.97)	0.70 (0.53, 0.93)	0.74 (0.53, 0.99)
Goldberg	0.58 (0.43, 0.78)	0.73 (0.54, 1.00)	0.70 (0.50, 0.96)	0.76 (0.53, 1.13)	0.70 (0.50, 0.97)	0.77 (0.52, 1.12)
Flavan-3-ol						
Sex-specific	1.09 (0.86, 1.41)	1.14 (0.89, 1.46)	1.15 (0.89, 1.48)	1.19 (0.93, 1.53)	1.09 (0.84, 1.41)	1.13 (0.87, 1.47)
Goldberg	1.05 (0.80, 1.39)	1.08 (0.82, 1.44)	1.09 (0.82, 1.45)	1.14 (0.86, 1.52)	1.03 (0.77, 1.37)	1.07 (0.80, 1.44)
Flavanone						
Sex-specific	0.81 (0.63, 1.04)	0.93 (0.72, 1.20)	0.91 (0.70, 1.18)	1.02 (0.71, 1.48)	0.93 (0.72, 1.22)	1.07 (0.73, 1.56)
Goldberg	0.88 (0.66, 1.17)	0.99 (0.74, 1.32)	0.98 (0.73, 1.31)	1.07 (0.70, 1.63)	0.99 (0.73, 1.34)	1.11 (0.72, 1.72)
Flavone						
Sex-specific	0.75 (0.58, 0.99)	0.96 (0.73, 1.26)	0.93 (0.70, 1.23)	1.28 (0.90, 1.82)	0.90 (0.67, 1.20)	1.18 (0.82, 1.69)
Goldberg	0.70 (0.50, 0.96)	0.87 (0.62, 1.21)	0.85 (0.60, 1.19)	1.15 (0.76, 1.77)	0.81 (0.57, 1.15)	1.07 (0.69, 1.66)
Flavonol						
Sex-specific	0.83 (0.64, 1.06)	1.00 (0.77, 1.29)	0.97 (0.75, 1.26)	1.23 (0.92, 1.64)	0.94 (0.72, 1.24)	1.14 (0.85, 1.55)
Goldberg	0.77 (0.57, 1.04)	0.91 (0.66, 1.24)	0.89 (0.65, 1.22)	1.12 (0.78, 1.59)	0.84 (0.61, 1.17)	1.02 (0.70, 1.47)
Isoflavone						
Sex-specific	0.65 (0.50, 0.84)	0.81 (0.62, 1.05)	0.80 (0.62, 1.05)	0.94 (0.70, 1.26)	0.87 (0.66, 1.14)	0.98 (0.73, 1.33)
Goldberg	0.70 (0.53, 0.93)	0.84 (0.63, 1.12)	0.84 (0.63, 1.11)	0.99 (0.72, 1.35)	0.88 (0.65, 1.18)	1.03 (0.74, 1.43)
Proanthocyanidins						
Sex-specific	0.51 (0.40, 0.67)	0.62 (0.48, 0.80)	0.62 (0.48, 0.81)	0.65 (0.49, 0.87)	0.65 (0.50, 0.85)	0.68 (0.50, 0.91)
Goldberg	0.48 (0.36, 0.65)	0.56 (0.41, 0.76)	0.56 (0.41, 0.76)	0.60 (0.43, 0.84)	0.58 (0.42, 0.79)	0.61 (0.43, 0.87)
Total flavonoid II[5]						
Sex-specific	0.87 (0.68, 1.11)	0.97 (0.76, 1.24)	0.97 (0.76, 1.25)	1.06 (0.82, 1.38)	0.92 (0.82, 1.19)	0.99 (0.76, 1.30)
Goldberg	0.77 (0.57, 1.03)	0.84 (0.63, 1.13)	0.85 (0.63, 1.14)	0.94 (0.69, 1.28)	0.79 (0.58, 1.07)	0.86 (0.63, 1.19)

1. REasons for Geographic and Racial Disparities in Stroke study (REGARDS). Hazard ratios (95% CI) were calculated with the use of Cox proportional hazards models.

2. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

3. sex-specific calorie restrictions: 800-4200 kcal/day for men and 500-3500 kcal/day for women

4. Using Goldberg cut-offs, where energy underreporters (n=5,647) and overreporters (n=15) were excluded, metabolic rate was estimated using the Schofield equation for height, weight and age, and physical activity level was estimated to be 1.55.

5. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, isoflavone and proanthocyanidin intake.

6. Model 1 = Adjusted for age, calorie intake and sex

7. Model 2= Model 1 + self-reported race, region of residense, educational attainment, household income, physical activity smoking status

8. Model 3= Model 2 + percent calories consumed from sweetened foods

9. Model 4 = Model 3 + beer and liquor intake, omega-3 fatty acids, vitamin C, vitamin E, folate, sodium, potassium, magnesium, supplement usage and fiber

10. Model 5= Model 3 + BMI, aspirin use, history of or use of medications for hypertension, hyperlipidemia or diabetes

11. Model 6 = Model 4 + BMI, aspirin use, history of or use of medications for hypertension, hyperlipidemia or diabet

Table S.5.3. Hazard ratios and 95% confidence interval for incident coronary heart disease by quintile of flavonoid intake for participants in the REGARDS[1] study, stratified by race.

		White (n=10,999)			Black (n=5,679)		
Quintile	Median Intake (range) mg/d	CHD events no.	Model I[4]	Model II[5]	CHD events no.	Model I	Model II
Total Flavonoid I[2]							
1	46 (≤ 72)	67	1.00 (-)	1.00 (-)	66	1.00 (-)	1.00 (-)
2	92 (73-113)	84	1.38 (0.99, 1.93)	1.48 (1.06, 2.07)	35	0.50 (0.32, 0.76)	0.54 (0.35, 0.83)
3	141 (114-182)	71	1.11 (0.79, 1.56)	1.23 (0.87, 1.73)	29	0.41 (0.26, 0.65)	0.46 (0.29, 0.73)
4	251 (183-352)	69	0.97 (0.69,1.37)	1.05 (0.75, 1.48)	39	0.64 (0.43, 0.96)	0.71 (0.47, 1.07)
5	599 (≥ 353)	102	1.35 (0.99,1.84)	1.43 (1.04, 1.95)	27	0.74 (0.47, 1.17)	0.82 (0.52, 1.29)
P-trend[3]			0.66	0.88		0.58	0.72
Anthocyanidin							
1	4.0 (≤ 5.8)	104	1.00 (-)	1.00 (-)	55	1.00 (-)	1.00 (-)
2	7.3 (5.9-8.7)	79	0.78 (0.58, 1.06)	0.84 (0.62, 1.14)	46	0.76 (0.50, 1.15)	0.78 (0.52, 1.18)
3	10.3 (8.8-12.2)	79	0.79 (0.58, 1.07)	0.90 (0.66, 1.23)	37	0.65 (0.42, 1.00)	0.71 (0.46, 1.10)
4	14.8 (12.3-18.5)	63	0.60 (0.44, 0.83)	0.72 (0.51, 1.00)	37	0.64 (0.42, 0.99)	0.71 (0.46,1.10)
5	24.7 (≥ 18.6)	68	0.56 (0.41, 0.77)	0.69 (0.49, 0.96)	21	0.47 (0.28, 0.78)	0.54 (0.32, 0.92)
P-trend			<0.001	0.03		<0.001	0.04
Flavan-3-ol							
1	8 (≤ 25)	75	1.00 (-)	1.00 (-)	59	1.00 (-)	1.00 (-)
2	38 (26-50)	64	1.04 (0.73, 1.48)	1.06 (0.74, 1.51)	41	0.63 (0.41, 0.99)	0.66 (0.43, 1.03)
3	68 (51-112)	78	1.38 (0.99, 1.94)	1.41 (1.00, 1.97)	33	0.55 (0.35, 0.89)	0.60 (0.38, 0.97)
4	187 (113-278)	74	1.02 (0.74, 1.42)	1.03 (0.74, 1.44)	36	0.71 (0.46, 1.11)	0.76 (0.49, 1.18)
5	529 (≥ 279)	102	1.32 (0.97, 1.80)	1.31 (0.96, 1.78)	27	0.92 (0.58, 1.46)	0.96 (0.60, 1.54)
P-trend			0.40	0.17		0.82	0.59
Flavanone							
1	2 (≤ 10)	99	1.00 (-)	1.00 (-)	32	1.00 (-)	1.00 (-)
2	9 (10–14)	84	1.05 (0.77, 1.43)	1.09 (0.80, 1.48)	27	0.67 (0.39, 1.15)	0.69 (0.41, 1.19)
3	17 (15–19)	54	0.71 (0.50, 0.99)	0.78 (0.56, 1.10)	39	0.82 (0.51, 1.33)	0.87 (0.53, 1.41)
4	34 (20–46)	79	0.93 (0.69, 1.26)	1.07 (0.79, 1.45)	50	0.93 (0.59, 1.47)	1.02 (0.64, 1.62)
5	58 (≥ 46)	77	0.76 (0.56, 1.03)	0.90 (0.66, 1.23)	48	0.79 (0.50, 1.25)	0.86 (0.54, 1.38)
P-trend			0.10	0.60		0.54	0.68

Flavone							
1	0.4 (≤ 0.51)	79	1.00 (-)	1.00 (-)	62	1.00 (-)	1.00 (-)
2	0.60 (0.52–0.69)	73	0.97 0.70, 1.34	1.06 (0.76, 1.46)	43	0.77 (0.51, 1.15)	0.85 (0.57, 1.28)
3	0.80 (0.70–0.94)	88	1.15 (0.85, 1.58)	1.31 (0.95, 1.80)	42	0.92 (0.62, 1.39)	1.05 (0.69, 1.58)
4	1.10 (0.95-1.45)	83	1.00 (0.73, 1.36)	1.19 (0.86, 1.64)	30	0.81 (0.52, 1.26)	0.99 (0.62, 1.56)
5	2.09 (≥ 1.46)	70	0.88 (0.63, 1.22)	1.04 (0.74, 1.47)	19	0.62 (0.37, 1.04)	0.76 (0.45, 1.30)
P-trend			0.02	0.88		0.03	0.43
Flavonol							
1	7 (≤ 9)	76	1.00 (-)	1.00 (-)	67	1.00 (-)	1.00 (-)
2	11 (9-14)	80	0.94 (0.69, 1.29)	0.99 (0.72, 1.37)	48	0.74 (0.50, 1.08)	0.80 (0.54, 1.17)
3	16 (14-19)	63	0.82 (0.58, 1.15)	0.91 (0.64, 1.28)	35	0.76 (0.50, 1.15)	0.82 (0.54, 1.25)
4	23 (19–28)	87	0.97 (0.71, 1.32)	1.08 (0.79, 1.49)	25	0.55 (0.35, 0.87)	0.61 (0.38, 0.98)
5	36 (≥ 28)	87	0.94 (0.69, 1.29)	1.05 (0.76, 1.44)	21	0.79 (0.48, 1.30)	0.96 (0.58, 1.59)
P-trend			0.32	0.68		0.04	0.41
Isoflavone							
1	0.1 (≤ 0.27)	105	1.00 (-)	1.00 (-)	60	1.00 (-)	1.00 (-)
2	0.39 (0.28-0.49)	75	0.75 (0.55, 1.03)	0.80 (0.58, 1.10)	37	0.57 (0.37, 0.89)	0.60 (0.38, 0.94)
3	0.57 (0.50–0.67)	69	0.76 (0.54, 1.07)	0.83 (0.59, 1.16)	34	0.51 (0.31, 0.83)	0.56 (0.34, 0.91)
4	0.79 (0.68–0.99)	78	0.91 (0.66, 1.25)	1.03 (0.74, 1.43)	37	0.67 (0.42, 1.08)	0.75 (0.47, 1.21)
5	1.55 (≥ 1.00)	66	0.73 (0.53, 1.01)	0.92 (0.66, 1.27)	28	0.51 (0.32, 0.82)	0.63 (0.39, 1.01)
P-trend			0.45	0.34		0.01	0.17
Proanthocyanidin							
1	42 (≤ 59)	106	1.00 (-)	1.00 (-)	66	1.00 (-)	1.00 (-)
2	70 (60-81)	75	0.70 (0.52, 0.95)	0.75 (0.55, 1.02)	41	0.66 (0.44, 0.98)	0.67 (0.45, 1.01)
3	91 (82-103)	64	0.60 (0.44, 0.83)	0.68 (0.49, 0.93)	33	0.57 (0.37, 0.88)	0.65 (0.42, 1.01)
4	117 (104-136)	84	0.74 (0.55, 0.99)	0.87 (0.64, 1.17)	30	0.58 (0.37, 0.90)	0.67 (0.43, 1.05)
5	168 (≥ 137)	64	0.54 (0.40, 0.74)	0.65 (0.47, 0.89)	26	0.48 (0.31, 0.76)	0.57 (0.36, 0.91)
P-trend			<0.001	0.03		<0.001	0.02
Total Flavonoid II[6]							
1	108 (≤ 149)	79	1.00 (-)	1.00 (-)	63	1.00 (-)	1.00 (-)
2	180 (150-212)	75	0.75 (0.55, 1.03)	1.09 (0.79, 1.50)	45	0.67 (0.45, 1.00)	0.75 (0.50, 1.11)
3	250 (213-298)	71	0.76 (0.54,1.07)	1.03 (0.74, 1.43)	27	0.44 (0.28, 0.70)	0.50 (0.31, 0.79)
4	367 (299-478)	71	0.91 (0.66,1.25)	0.94 (0.68, 1.31)	39	0.70 (0.46, 1.05)	0.82 (0.55, 1.24)
5	717 (≥ 479)	97	0.73 (0.53, 1.01)	1.15 (0.85, 1.56)	22	0.63 (0.39, 1.03)	0.71 (0.43, 1.15)
P-trend			0.69	0.39		0.08	0.34

1. REasons for Geographic and Racial Disparities in Stroke study (REGARDS). Hazard ratios (95% CI) were calculated with the use of Cox proportional hazards models.
2. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes
3. P-trend values were calculated with the use of the Wald test.
4. Model I: adjusted for age, energy and sex
5. Model II: model adjusted for age, energy, sex, region of residence, educational attainment, household income, exercise, smoking status, percent calories from sweetened foods
6. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, isoflavone and proanthocyanidin intakes

Table S.5.4. Hazard ratios and 95% confidence interval for incident acute ischemic stroke by quintile of flavonoid intake for participants in the REGARDS[1] study, stratified by sex.

		Men (n=6,880)			Women (n=9,798)		
Quintile	Median Intake (range) mg/d	CHD events No.	Model I[4]	Model II[5]	CHD events No.	Model I	Model II
Total Flavonoid I[2]							
1	46 (≤ 72)	83	1.00 (-)	1.00 (-)	50	1.00 (-)	1.00 (-)
2	92 (73-113)	70	1.07 (0.77, 1.48)	1.18 (0.85, 1.65)	49	0.69 (0.46, 1.05)	0.74 (0.49, 1.11)
3	141 (114-182)	67	1.03 (0.74, 1.44)	1.17 (0.84, 1.64)	33	0.43 (0.27, 0.68)	0.46 (0.29, 0.73)
4	251 (183-352)	67	0.97 (0.70, 1.35)	1.06 (0.77, 1.47)	41	0.54 (0.35, 0.82)	0.61 (0.40, 0.93)
5	599 (≥ 353)	81	1.28 (0.94, 1.74)	1.38 (1.01, 1.89)	48	0.65 (0.43, 0.97)	0.77 (0.51, 1.15)
P-trend[3]			0.11	0.08		0.42	0.93
Anthocyanidin							
1	4.0 (≤ 5.8)	114	1.00 (-)	1.00 (-)	45	1.00 (-)	1.00 (-)
2	7.3 (5.9-8.7)	81	0.82 (0.61, 1.10)	0.88 (0.65, 1.18)	44	0.72 (0.47, 1.11)	0.73 (0.47, 1.13)
3	10.3 (8.8-12.2)	69	0.77 (0.57, 1.05)	0.91 (0.67, 1.25)	47	0.70 (0.46, 1.07)	0.74 (0.48, 1.13)
4	14.8 (12.3-18.5)	54	0.63 (0.45, 0.87)	0.75 (0.54, 1.05)	46	0.61 (0.40, 0.93)	0.68 (0.44, 1.06)
5	24.7 (≥ 18.6)	50	0.54 (0.38, 0.75)	0.68 (0.47, 0.96)	39	0.52 (0.34, 0.81)	0.63 (0.40, 0.99)
P-trend			<0.001	0.02		0.008	0.11
Flavan-3-ol							
1	8 (≤ 25)	89	1.00 (-)	1.00 (-)	45	1.00 (-)	1.00 (-)
2	38 (26-50)	66	1.00 (0.71, 1.41)	1.04 (0.74, 1.47)	39	0.63 (0.39, 1.00)	0.66 (0.41, 1.05)
3	68 (51-112)	60	1.10 (0.78, 1.56)	1.16 (0.82, 1.63)	51	0.79 (0.50, 1.23)	0.83 (0.53, 1.30)
4	187 (113-278)	71	1.04 (0.76, 1.43)	1.06 (0.77, 1.46)	39	0.61 (0.39, 0.96)	0.68 (0.43, 1.07)
5	529 (≥ 279)	82	1.31 (0.97, 1.78)	1.32 (0.97, 1.80)	47	0.75 (0.49, 1.15)	0.87 (0.57, 1.34)
P-trend			0.12	0.14		0.64	0.80
Flavanone							
1	2 (≤ 10)	87	1.00 (-)	1.00 (-)	44	1.00 (-)	1.00 (-)
2	9 (10–14)	63	0.94 (0.67, 1.32)	0.96 (0.69, 1.36)	48	0.91 (0.59, 1.41)	0.92 (0.59, 1.42)
3	17 (15–19)	59	0.87 (0.62, 1.21)	0.97 (0.69, 1.37)	34	0.63 (0.40, 1.01)	0.63 (0.39, 1.00)
4	34 (20–46)	80	1.03 (0.76, 1.41)	1.20 (0.88, 1.64)	49	0.84 (0.55, 1.27)	0.87 (0.57, 1.33)
5	58 (≥ 46)	79	0.86 (0.63, 1.18)	1.04 (0.76, 1.44)	46	0.70 (0.46, 1.08)	0.70 (0.45, 1.08)
P-trend			0.59	0.46		0.19	0.22

Flavone							
1	0.4 (≤ 0.51)	95	1.00 (-)	1.00 (-)	44	1.00 (-)	1.00 (-)
2	0.60 (0.52–0.69)	74	0.91 (0.67, 1.24)	1.02 (0.74, 1.39)	48	0.78 (0.51, 1.20)	0.83 (0.54, 1.28)
3	0.80 (0.70–0.94)	83	1.19 (0.88, 1.61)	1.39 (1.03, 1.89)	34	0.78 (0.52, 1.19)	0.89 (0.58, 1.36)
4	1.10 (0.95-1.45)	61	0.95 (0.68, 1.31)	1.18 (0.85, 1.65)	49	0.76 (0.50, 1.13)	0.90 (0.59, 1.38)
5	2.09 (≥ 1.46)	55	0.97 (0.70, 1.35)	1.19 (0.84, 1.69)	46	0.50 (0.32, 0.78)	0.61 (0.38, 0.99)
P-trend			0.91	0.19		0.01	0.14
Flavonol							
1	7 (≤ 9)	93	1.00 (-)	1.00 (-)	50	1.00 (-)	1.00 (-)
2	11 (9-14)	79	0.87 (0.65, 1.18)	0.96 (0.70, 1.30)	49	0.76 (0.51, 1.13)	0.79 (0.53, 1.19)
3	16 (14-19)	57	0.81 (0.58, 1.13)	0.92 (0.66, 1.29)	41	0.68 (0.44, 1.03)	0.75 (0.49, 1.15)
4	23 (19–28)	75	0.96 (0.71, 1.30)	1.08 (0.79, 1.48)	37	0.55 (0.36, 0.85)	0.64 (0.41, 1.00)
5	36 (≥ 28)	64	0.97 (0.71, 1.34)	1.12 (0.81, 1.56)	44	0.63 (0.42, 0.94)	0.78 (0.51, 1.19)
P-trend			0.61	0.20		0.04	0.32
Isoflavone							
1	0.1 (≤ 0.27)	109	1.00 (-)	1.00 (-)	56	1.00 (-)	1.00 (-)
2	0.39 (0.28-0.49)	77	0.79 (0.58, 1.08)	0.84 (0.62, 1.16)	35	0.49 (0.32, 0.77)	0.52 (0.33, 0.82)
3	0.57 (0.50–0.67)	56	0.68 (0.48, 0.97)	0.75 (0.52, 1.07)	47	0.60 (0.38, 0.94)	0.65 (0.41, 1.02)
4	0.79 (0.68–0.99)	67	0.95 (0.68, 1.32)	1.06 (0.76, 1.48)	48	0.62 (0.40, 0.97)	0.72 (0.46, 1.13)
5	1.55 (≥ 1.00)	59	0.74 (0.53, 1.02)	0.93 (0.67, 1.29)	35	0.50 (0.32, 0.77)	0.62 (0.40, 0.98)
P-trend			0.04	0.87		0.11	0.17
Proanthocyanidin							
1	42 (≤ 59)	120	1.00 (-)	1.00 (-)	52	1.00 (-)	1.00 (-)
2	70 (60-81)	70	0.67 (0.49, 0.90)	0.71 (0.53, 0.97)	46	0.69 (0.45, 1.04)	0.73 (0.48, 1.11)
3	91 (82-103)	59	0.65 (0.47, 0.89)	0.72 (0.52, 0.99)	38	0.49 (0.32, 0.75)	0.58 (0.37, 0.90)
4	117 (104-136)	63	0.70 (0.52, 0.96)	0.82 (0.60, 1.12)	51	0.62 (0.42, 0.93)	0.78 (0.52, 1.18)
5	168 (≥ 137)	56	0.61 (0.44, 0.84)	0.72 (0.52, 1.00)	34	0.39 (0.25, 0.60)	0.51 (0.33, 0.79)
P-trend			0.003	0.09		<0.001	0.01
Total Flavonoid II[6]							
1	108 (≤ 149)	89	1.00 (-)	1.00 (-)	53	1.00 (-)	1.00 (-)
2	180 (150-212)	76	1.03 (0.76, 1.41)	1.12 (0.82, 1.53)	44	0.60 (0.40, 0.90)	0.64 (0.42, 0.97)
3	250 (213-298)	62	0.91 (0.66, 1.26)	1.04 (0.75, 1.44)	36	0.43 (0.28, 0.67)	0.48 (0.31, 0.75)
4	367 (299-478)	64	0.91 (0.66, 1.25)	1.02 (0.74, 1.42)	46	0.54 (0.36, 0.81)	0.65 (0.43, 0.97)
5	717 (≥ 479)	77	1.14 (0.84, 1.54)	1.23 (0.91, 1.68)	42	0.51 (0.34, 0.77)	0.63 (0.42, 0.96)
P-trend			0.45	0.37		0.04	0.27

1. REasons for Geographic and Racial Disparities in Stroke study (REGARDS). Hazard ratios (95% CI) were calculated with the use of Cox proportional hazards models.
2. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes
3. P-trend values were calculated with the use of the Wald test.
4. Model I: adjusted for age and energy
5. Model II: model adjusted for age, energy, sex, race, region of residence, educational attainment, household income, exercise, smoking status, history of coronary artery disease, age x race interaction term
6. Sum of anthocyanidin, flavan-3-ol, flavanone, flavone, flavonol, isoflavone and proanthocyanidin intakes

CHAPTER 6: DIETARY FLAVONOID INTAKE IS ASSOCIATED WITH COGNITIVE FUNCTION: THE REGARDS STUDY

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Abbreviations: AFT, Animal Fluency Test; CAD, coronary artery disease; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CI, confidence interval; FFQ, Food Frequency Questionnaire; FNDDS, Food and Nutrient Database for Dietary Studies; REGARDS, Reasons for Geographic and Racial Differences in Stroke; USDA, United States Department of Agriculture; WLL, Word List Learning; WLD, Word List Delayed Recall

Keywords: flavonoid, cognitive impairment, disparities

Abstract:

Flavonoids are dietary polyphenolic compounds with potential neuroprotective effects, but limited data are available on whether flavonoid intake is related to cognitive function. We assessed the association between total flavonoid and flavonoid subclass intakes and cognitive function in a biracial cohort. Participants were 14,370 black and white men and women, enrolled in the REGARDS study between 2003 and 2007, who completed a Block-98 food frequency questionnaire (FFQ) at enrollment. Flavonoid intake was estimated using USDA flavonoid databases. Learning, verbal memory and executive function were measured biennially and their standardized scores averaged to obtain a composite cognitive score. Results from linear mixed models indicated that intake in the highest quintile of anthocyanidin, flavone and flavonol, compared with the lowest quintile, was associated with higher cognitive function. These results were equivalent to 2-3 years of younger age, both at baseline and at follow-up, though neither anthocyanidin, flavones nor flavonol intakes were associated with a change in the rate of cognitive decline. Although our data do not prove a causal association, it suggests that dietary anthocyanidins, flavones and flavonols may promote healthy cognitive function.

Introduction:

As the population ages, the worldwide prevalence of dementia is projected to double every 20 years reaching roughly 115 million cases by 2050 [3]. Interventions that delay the onset and progression of dementia by just one year, could reduce its prevalence at the population level by about 9.2 million fewer cases worldwide by 2050 [275]. With no curative treatment options and only moderately effective symptomatic therapies available, preventive strategies such as dietary modification may be beneficial. Flavonoids are bioactive, polyphenolic compounds, found ubiquitously in plant-based foods, including fruits, vegetables, tea, and wine. Evidence suggests that flavonoids can cross the blood-brain barrier, concentrating in areas involved in learning and memory such as the hippocampus [259,276,277]. At low, physiologic concentrations, these compounds interact with neuronal intracellular signaling pathways mediating neuroinflammation, neurodegeneration, and neurogenesis [278]. Flavonoids also possess cardioprotective effects [14] and appear to reduce the risk of cardiovascular disease [13]. Considering that vascular risk factors predict poor cognitive health [279], it is also possible that flavonoids may exert beneficial effects on cognitive health through vascular mechanisms.

Short-term supplementation trials of flavonoid-rich foods in trials in older adults have shown improvements in cognitive function [162,280,281]. However, since cognitive function declines over years to decades, studies of long-term dietary habits are likely most relevant to cognitive health. Few epidemiologic studies have examined flavonoids in habitual diet in relation to cognitive function [166,167,282,283]. The single epidemiologic study in the U.S. that has explored these associations included predominantly white, female, health-care providers [167]. Evidence from a geographically diverse U.S. cohort, including men and non-Hispanic blacks is lacking in spite of known racial disparities in fruit, vegetable and flavonoid consumption [32-35] and racial and

regional disparities in cognitive impairment [30,284]. Incident cognitive impairment in stroke-free participants is 18% higher in those who live in Southeastern United States compared to other regions [30]. Furthermore, this field of research has been hampered by a lack of comprehensive data for food flavonoid composition. Missing values for flavonoids in some foods, inadequate adjustment for proportionate intake of flavonoids from mixed dishes and lack of adjustment for food processing losses may have led to measurement error when estimating flavonoid intake. To address this issue, in 2014, the U.S. Department of Agriculture (USDA) released the Provisional Flavonoid Addendum [25]. Using this new, expanded flavonoid database, we evaluated the longitudinal associations of total dietary flavonoid and seven flavonoid subclass intakes with cognitive function in a geographically diverse biracial cohort study.

Methods

The REasons for Geographic and Racial Difference in Stroke (REGARDS) study is a prospective cohort study designed to study racial and geographic differences in stroke mortality. Between 2003 and 2007, 30,239 community-dwelling adults, ≥45 years old, living in the continental United States, were recruited into the study. Non-Hispanic white and black participants were recruited by mail and telephone contacts using commercially available lists. Participants were oversampled from the Southeastern region of the U.S., referred to as the "Stroke Belt", including Alabama, Arkansas, Georgia, Louisiana, Mississippi, Tennessee, North Carolina and South Carolina. Self-reported race and sex were balanced by design resulting in a cohort with 56% stroke-belt residents and 44% residents of the remaining contiguous lower 48 states, 42% black and 55% female participants [174]. Baseline data about health status and medical history, including stroke risk factors, were collected by computer-assisted telephone interview (CATI). Trained healthcare professionals conducted in-home visits to obtain written consent,

height, weight, blood pressure measurements, resting electrocardiogram, a medication inventory and collect blood and urine samples. The institutional review boards of all participating institutions approved the study, and all participants provided written informed consent.

Cognitive assessment

Formally trained and certified REGARDS telephone interviewers administered telephone-based computer-assisted assessments of cognitive function. Cognitive assessments were conducted longitudinally and REGARDS study personnel continually monitored interviewer performance. A biennial, cognitive test battery was added to study telephone follow-up interviews in 2006, once study follow-up for stroke endpoints had started. The battery included the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Word List Learning (WLL), Word List Recall (WLD) [245] and Animal Fluency (AFT) [246]. CERAD WLL measures new learning (scores range 0-30) and the WLD measures verbal memory (scores range 0-10). Immediate and delayed recalls are associated with Alzheimer's and frontotemporal dementia [285]. The AFT assesses executive function, with scores representing the number of animals named in 60 seconds with no upper score bound. In all cases, a higher score is favorable. Executive function is impaired in mild cognitive impairment (MCI) and may improve screening accuracy of the Mini Mental State exam for MCI and non-Alzheimer's disease dementia [286,287]. Telephone assessments of global cognitive function, verbal memory and verbal fluency are valid and reliable in middle-aged and older adults [288-290]. These cognitive measures are consistent with the Vascular Cognitive Impairment Harmonization Standards and have been validated for black and white individuals [291,292]. Because the cognitive tests were scaled differently, we used z-scores to create a composite cognitive score. Similar methods have been used previously[12,167]. Specifically, we averaged together z-scores for all available cognitive scores at each cognitive assessment time period.

Dietary Assessment

A self-administered Block 98 Food Frequency Questionnaire (FFQ) was left with study participants at the in-home visit at study enrollment and was returned by mail to the REGARDS coordinating center. The Block 98 FFQ is a 107-item questionnaire, developed by NutritionQuest (Berkeley, CA), has been validated in populations similar to REGARDS [213,214]. Though not specifically designed to measure flavonoid intake, the FFQ does inquire about intakes of foods with high flavonoid content over the previous year, including fruits, vegetables, tea, and wine.

Flavonoid Intake

Flavonoid intakes of interest were total flavonoid intake, anthocyanidins, flavan-3-ols, flavanones, flavone, flavonols, isoflavones, and proanthocyanidins, which are polymers of flavan-3-ols. Two complementary USDA databases, the Provisional Flavonoid Addendum and the USDA Database for the Proanthocyanidin Content of Selected Foods, provided flavonoid values used to estimate flavonoid intake [25,46]. **Table S.6.1** summarizes the flavonoid compounds included in each database and some of their major food sources. The newly-released Provisional Flavonoid Addendum, a comprehensive food flavonoid composition database, contains data for 29 flavonoids in six flavonoid subclasses for 7,174 foods/beverages in the USDA's Food and Nutrient Database for Dietary Studies (FNDDS), 4.1 and accounts for processing and cooking effects on flavonoids better than previous USDA flavonoid databases [25]. The Provisional Flavonoid Addendum and Proanthocyanidin Database contain overlapping information about flavan-3-ols, identified as monomers in the Proanthocyanidin Database. When a food was included in both databases, and if a flavan-3-ol value (Addendum), and monomer value (Proanthocyanidin Database) were both available, only the flavan-3-ol value from the Provisional Flavonoid Addendum was used.

Food items on the Block 98 FFQ were linked to the Provisional Flavonoid Addendum and Proanthocyanidin Database using 8-digit FNDDS and 5-digit Standard Reference food codes, respectively. The sum of flavonoid compounds was calculated for each flavonoid subclass. For combined items on the FFQ, such as "apples or pears," a weighted average of flavonoids was calculated using population-based weighted intakes, consistent with the Block 98 FFQ. Estimated daily flavonoid intake for each participant was calculated by multiplying the reported amount (grams) of food consumed by the flavonoid content of the corresponding food (mg flavonoid/100 grams of food) and summed across foods.

Statistical analysis

Participants in these analyses included only those who, at enrollment, had no history of stroke, did not have missing data on key covariates and had adequately completed the FFQ. Completion of the FFQ was considered adequate if at least 85% of the FFQ was answered and if reported daily calorie intake was between 500 and 5,000 calories. For longitudinal analysis, we also excluded those who did not have at least two cognitive assessments of the same test, WLL, WLD or AF.

We created quintiles of reported total and subclass flavonoid intakes defined from the total study population because gender-specific intakes were not meaningfully different. Given methodological differences in the creation of the Provisional Flavonoid Addendum and the Proanthocyanidin Database, we examined two total flavonoid intake variables, one including only flavonoid values found in the Provisional Flavonoid Database (total flavonoid I) and another adding proanthocyanidin intake (total flavonoid intake II). Nutrient values were energy adjusted using the residual method [271]. We compared demographic, socioeconomic, behavioral, and nutrient characteristics by quintile of total flavonoid intake using ANOVA and χ^2- tests

according to variable distribution. We also examined the distribution of self-reported race and region of residence by quintile of flavonoid subclass intakes.

Flavonoid-based analyses

To assess the cross-sectional association between flavonoid intake and global cognitive performance, we used simple linear regression to obtain effect estimates. Models were built sequentially, beginning with an unadjusted model (model 0). Model 1 included continuous age, continuous energy intake and sex. In model 2 demographic variables were added including self-reported race (non-Hispanic black or white) and region of residence (Stroke Belt, non-Stroke Belt). Model 3 included yearly household income (categorized as <$20,000, $20,000-34,999, $35,000-74,999, ≥$75,000, or refused) and education (categorized as < HS, high school, some college, college and above). Model 4 included health behaviors (physical activity and smoking status) and presence of depressive symptoms at baseline. Physical activity was categorized as none, 1-3 times/week, or ≥4 times/week and smoking status was categorized as never, former or current. In additional analyses we added dietary variables to the models, including whole grains, fatty acids, fiber, folate, vitamin C, β-carotene, alcohol, sodium and percent energy from sweetened foods and beverages (sweetened cereals and beverages, jelly, and sugar/honey added to coffee or tea). We also considered nutrients derived from supplements but this did not change estimates. Because dietary variables can be strongly intercorrelated, they were added to the model individually and retained if they were independently associated with cognitive performance or changed the HR in the top quintile of exposures greater than 10%. Covariates included in the final, parsimonious model included age, total energy intake, sex, as well as self-identified race, region of residence, socioeconomic and health behavioral factors, the presence of depressive symptoms and percent of energy consumed from sweetened foods and beverages.

Linear mixed models were used to evaluate the association between flavonoid intake and changes in the composite cognitive score over time, allowing for correlation within participant cognitive measures over time and allowing for varying numbers of cognitive assessments. We also tested adjusted differences in the composite cognitive score at each measurement time point. All models included a term for flavonoid intake, time and the interaction between flavonoid intake and time. Time was modeled as a nominal variable (reflecting the cognitive assessment occasion), as opposed to a continuous variable, to avoid assumptions about shape of cognitive function trends over time. The β-coefficient for interaction terms with time represents the estimated effect of the variables on the rate of change in cognitive score. A positive β-coefficient for interaction terms with time and flavonoid intake reflects a decrease in the rate of decline with higher flavonoid intake. Covariate adjustment was conducted as in the cross-sectional analysis. Effect modification by age (dichotomized at 65 years), sex, self-reported race, and region of residence was examined by modeling 2- and 3-factor interaction terms among covariates (age, sex, race and region of residence), flavonoid intake and time. Lower-order terms were retained as needed to maintain hierarchically well-formulated models. Several covariance structures were compared based on prior knowledge of cognitive measures and the unstructured covariate structure was selected based on biologic plausibility and the lowest Akaike information criterion.

Food-based analyses

We also examined the association between selected FFQ food items and cognitive function using multivariable models similar to those used for flavonoid intake. We included foods if they comprised at least 1% of reported total flavonoid intake or if the food was previously determined, in scientific literature, to be associated with cognitive health. These foods included tea (52% of total flavonoid intake), apples or pears (6%), cakes (5%), citrus fruits/juices (5%),

canned fruit (3%), wine (3%), 100% fruit juice excluding orange (3%), peanuts/nuts (2%), legumes (2%), berries (1%), cocoa (1%) and tofu (1%). **Table S.6.2** shows the primary food sources of flavonoids in the REGARDS cohort. Meaningful categories of food consumption were created as follows. Citrus fruit/juices, apples/pears, legumes and 100% fruit juice (excluding orange juice) were categorized as <1, 1-6, and ≥ 7 servings/week. Berry consumption was categorized as <1, 1-3, and ≥ 4 servings/month. Wine was categorized as nondrinkers, ≤1 drink/wk, 2-4 drinks/wk, and ≥5 drinks/wk. Chocolate, peanut/nuts, tea and tofu intake were categorized as consumers versus non-consumers.

Sensitivity analyses

Sensitivity analysis using multiply-imputed missing covariates was conducted to assess possible bias due to exclusion of participants with missing information on key covariates. We also repeated our analyses excluding participants in the bottom 10% of the initial cognitive tests, because those participants may have already had early stage dementia, which might cause reductions in fruit and vegetable intake. Finally, we used a stricter definition of the average composite cognitive score, requiring that the participant have z-scores for each of the three cognitive tests (WLL, WLD, and AF), rather than requiring at least one z-score to calculate the average. We also assessed the longitudinal associations between flavonoid intake and each of the three cognitive tests separately.

Results

There were 21,636 participants in the dietary subsample. Participants who did not return the FFQ were more often black (61.3% versus 38.7%), less educated (20% versus 10% with less than high school education) and had lower income (24.0% versus 15.8% earned less than $20,000). After excluding 1,223 participants with a history of stroke at study enrollment and 343 participants

missing data on key covariates, there were 17,509 participants available for the cross-sectional analysis. Of these, 2,815 participants who did not have ≥ 2 AFT, WLL or WLD assessments over time were excluded from the longitudinal analysis, leaving 14,370 participants available for the analysis. A flow diagram for study participation is shown in **figure 6.1.** In the longitudinal analysis, the number of participants contributing cognitive scores at each measurement occasion decreased over time (Time 0: n=14,186, Time 1: n=14,308, Time 2: n=11,176, Time 3: n=6,971, and Time 4: n=1,460).

Mean and median total flavonoid intakes for men were 231 mg and 133 mg, respectively; corresponding values for women were 236 mg and 133 mg. Baseline characteristics by quintiles of energy-adjusted total flavonoid intake I are summarized in **table 6.1** and did not differ using total flavonoid intake II (total flavonoid I plus proanthocyanidin). Individuals with higher total flavonoid intake were more likely to be white, more educated and less sedentary, to have higher reported household income and to be nonsmokers.

Mean differences for the group effect for quintiles of flavonoid intake in the cross-sectional assessment are shown in **table 6.2**. Comparing extreme quintiles of flavonoid intake, there was a positive, statistically significant association between all flavonoid intake measures, in both minimally- (model 1) and fully-adjusted models (model 2), except for flavanones, proanthocyanidins and total flavonoids I and II. Positive mean differences indicate higher cognitive scores in those with greater flavonoid intake. The magnitudes of the estimated mean differences were attenuated after multivariable adjustment. Statistically significant, fully adjusted, extreme-quintile comparisons were accompanied by a statistically significant linear trend in all cases. Effect estimates from the cross-sectional analysis were not meaningfully different from the group effect estimates in the longitudinal analysis (data not shown). To aid

interpretation of results, we found that each additional year of age at enrollment was associated with a mean decrease of 0.036 standard units on the composite cognitive score. In comparison, the mean difference observed for extreme quintiles of flavone intake at the first cognitive assessment was equivalent to about 3 fewer years of age at enrollment. The adjusted mean differences for the group effect comparing extreme quintiles of flavonoid intake, obtained from longitudinal analysis, at each cognitive assessment occasion for anthocyanidins, flavones and flavonols are shown in **figure 6.2**. There was a statistically significant positive association over the first four cognitive assessments for flavones and over the first three cognitive assessments for flavonols and anthocyanidins. Adjusted mean differences for other flavonoid subclasses over time were statistically significant only for the first cognitive assessment (except isoflavones over first two assessments) and are shown in **table S.6.3**. Participant numbers at the fifth cognitive assessment were significantly reduced leading to wide confidence intervals. There were no statistically significant two-way interactions for flavonoids and time or three-way interactions with flavonoids, covariates and time (all *P*-interaction > 0.10).

In food-based analyses, there were statistically significant positive associations between the consumption of several flavonoid-rich foods/beverages and cognitive measures, indicating higher cognitive scores with greater intakes **(table 6.3)**. There was a positive association between drinking wine and 100% fruit juice (excluding orange juice) and cognitive score, with no clear dose-dependent relationship. There was also a positive association for chocolate, peanut/tree nut, tea and tofu consumption. The positive association between berry consumption and the composite cognitive score was seen only in those who reported consuming at least four servings of berries per week. As in flavonoid models, we found that each additional year of age at enrollment was associated with a mean decrease of 0.036 standard units on the composite z-

score. The mean differences that we observed for extreme categories of wine intake were equivalent to roughly two years of age difference at study enrollment. Statistically significant mean differences were observed at the first four cognitive assessments for wine, 100% fruit juice, berries, chocolate, peanut/tree nuts, tea and tofu consumption (**table S.6.4**). Participant numbers at the fifth cognitive assessment were significantly reduced leading to wide confidence intervals. There were no statistically significant two-way interactions for foods and time or three-way interactions between foods, covariates and time (all p-interaction > 0.20). Results from sensitivity analyses, including multiple-imputation of missing covariates, excluding those with cognitive performance scores below the 10^{th} percentile and a more conservative definition of the composite score, were not materially different. In longitudinal analysis, when we considered each cognitive test separately, adjusted mean differences comparing the highest compared to the lowest intake were statistically significant for anthocyanidins, flavones and flavonols, consistent with the results using the composite cognitive score (see supplementary figures 1A-C).

Discussion

This is the first prospective cohort study to examine the associations between total flavonoid and flavonoid subclass intakes with cognitive function scores in a biracial national cohort, using the newly expanded USDA Provisional Flavonoid Addendum. We found meaningful adjusted mean differences in the composite cognitive scores at three to four cognitive assessment occasions for anthocyanidins, flavones and flavonols, though there was no difference in the rate of cognitive change comparing extreme quintiles of flavonoid intake. While we observed a group effect at the first cognitive assessment for all flavonoid measures, except total flavonoid I and flavanones, we consider our results most robust for anthocyanidins, flavones and flavonols because these persisted over at least three cognitive assessment occasions.

Early epidemiologic studies examining flavonoid intake and cognitive health often included only flavonols and flavones, rather than the wider variety of flavonoid subclasses available in flavonoid databases today. Using community-based samples in the Personnes Agées Quid (PAQUID) study in Bordeaux, France and The Rotterdam Study in Rotterdam, Netherlands, several studies reported protective associations between greater flavone and flavonol intake and rate of cognitive decline [166] and dementia risk [165,283] in participants who were at least 65 years old. The persistent positive associations for flavones and flavonols over multiple cognitive assessments in this study are consistent with the findings from these early studies, though we did not observe an association with change in rate of cognitive decline.

Recently, the Nurses' Health Study (NHS) used an expanded flavonoid composition tables that include more flavonoid subclasses to assess the association between flavonoid intake and cognitive decline. Greater total flavonoid, anthocyanidin, flavonol and berry intakes were associated with slower rates of cognitive decline in women who were at least 70 years old. Effect estimates were comparable to 1.5 to 2.5 years of cognitive aging [167]. Again, though we did not find a change in the rate of cognitive decline, the magnitudes of the group effects for anthocyanidin and berry intakes in this study are consistent with findings from NHS. Several differences between PAQUID and NHS, which reported a decrease in the rate of cognitive decline, and this study, which did not find a change in trajectory, may explain different results. PAQUID and NHS used more instruments to assess cognitive function and in PAQUID, psychometric testing was conducted in-person with neurologist confirmation of dementia if needed. These methodologic differences may have led to less measurement error of the outcome in PAQUID and NHS, yielding greater statistical power to detect statistically significant interaction. Furthermore, the mean and median participant age at enrollment in our study was 64

years old and eligibility began at 45 years, which is much younger than both the PAQUID study, which restricted age to ≥ 65 with an average age at enrollment of 77, and NHS, which restricted age to ≥ 70 and reported 74 years old as the average age for those in the analytic cohort. However, when analysis was restricted to those ≥ 65 years old at enrollment, our results were not meaningfully changed.

Another important difference between REGARDS and NHS study cohorts is the demographic distribution. By design, the REGARDS cohort oversampled non-Hispanic blacks and residents of the Stroke Belt, whereas participants in NHS were predominantly white and living outside of the Stroke Belt. The prevalence of cardiovascular risk factors is higher in black Americans and residents of the Stroke Belt. Moreover, black Americans also have a higher prevalence of vascular dementia than their white counterparts [293]. Given that vascular risk factors at midlife are associated with poor cognitive function in older age [279] and that flavonoids likely act through cardioprotective mechanisms, it is possible that the majority of the benefit from high flavonoid intake observed in this study was mediated by vascular health benefits in midlife, demonstrated as a persistent group effect for flavonoids, without a change in cognitive trajectory.

Associations between greater intake of wine, 100% fruit juice and berries and cognitive performance scores complement our flavonoid findings as these foods are typically rich in a variety of flavonoids, including anthocyanidins, flavones and flavonols, as well as others. Nuts, chocolate, tea and tofu are rich in flavonoid subclasses that were not associated with cognitive performance over time, in this study, such as flavan-3-ols, flavanones and isoflavones. Though these foods have all been favorably associated with cognitive function in the literature, most studies were intervention studies. It is possible that differences in study design explain inconsistent results for the representative flavonoids found in these foods, such as a threshold

effect resulting from higher concentrations of flavonoids in experimental conditions as compared to typical diets.

Strengths of this study include the large, well-characterized, biracial, geographically diverse sample, prospective design and detailed cognitive measures. The flavonoid database used is the most comprehensive database available to assess flavonoid intake in the U.S. population. The ability to account for processing factors is an improvement in flavonoid exposure estimation. Our study also has limitations. First, dietary intake measurements were based on a FFQ administered at study enrollment but was not repeated during study follow-up. Thus, misclassification of dietary exposure may have occurred if participants' diets changed over time leading to bias toward the null and underestimation of the association, including the null association in the longitudinal cognitive function analysis. Additional measurement error may have occurred since FFQs cannot capture all potential dietary sources of flavonoids. However, by using a comprehensive flavonoid database and detailed FFQ, the most important dietary sources of flavonoids were likely captured. Also, the flavonoid content of plants varies depending on cultivars, growing conditions and storage. However, the flavonoid values used in this study were mean values for foods, which are the most appropriate measure to use in a large epidemiologic study such as this, considering that participants likely consumed various foods from various sources. Flavonoid studies that consider multiple flavonoid subclasses are inherently limited by multiple comparisons, therefore statistical significance should be interpreted cautiously and in the context of existing literature. The use of telephone cognitive assessments rather than in-person evaluation by a psychologist may have led to measurement error; yet telephone assessment of cognitive function has been validated [288-290] and has been used successfully to demonstrate an association between incident stroke and cognitive decline [294]. Only a small

sample of participants was available at the fifth cognitive assessment, however, this was a natural result of some participants entering the REGARDS study at the end of the enrollment period. Cognitive assessments are still ongoing in REGARDS, which will allow additional evaluation in the future. Finally, as in all observational studies, the possibility of residual confounding cannot be excluded. However, a wide range of potential confounders were considered in the modeling process and only those that yielded significant changes in the effect estimates, suggesting confounding, were retained in final models.

In conclusion, we found that greater consumption of anthocyanidins, flavones and flavonols, as well as several flavonoid-rich foods, is associated with better cognitive performance at multiple time points, though not associated with a change in the rate of cognitive decline. Associations did not differ by self-reported race or region of residence.

Take Away Points

Greater consumption of anthocyanidins, flavones and flavonols is associated with better cognitive performance at multiple time points, though is not associated with a change in the rate of cognitive decline.

Greater consumption of many flavonoid-rich foods is also associated with better cognitive performance at multiple time points.

While effect estimates for flavonoid intake are relatively small, these small mean differences in cognitive function may be important at a population level.

Table 6.1. Baseline characteristics for 17,509 participants with at least two composite cognitive scores in REGARDS by Quintile of Total Flavonoid Intake (total I)[1]

	Quintiles of Total Flavonoid I Intake				
	Q1	**Q2**	**Q3**	**Q4**	**Q5**
Characteristics	n=3501	n=3502	n=3502	n=3502	n=3502
Age, y (SD)	63.1 (9.2)	64.9 (9.2)	65.2 (8.8)	65.2 (9.3)	63.6 (8.8)
Female, n (%)	1544 (44.1)	2066 (59.0)	2147 (61.3)	2042 (58.3)	2038 (58.2)
White, n (%)	2230 (63.7)	2108 (60.2)	2150 (61.4)	2360 (67.4)	2816 (80.4)
Stroke Belt, n (%)	1807 (51.6)	1839 (52.5)	1856 (53.0)	2070 (59.1)	2287 (65.3)
Physical activity, n (%)					
None	1271 (36.3)	1180 (33.7)	1002 (28.6)	1058 (30.2)	1096 (31.3)
1-3 times/wk	1232 (35.2)	1310 (37.4)	1376 (39.3)	1345 (38.4)	1275 (36.4)
≥ 4 times/wk	1001 (28.6)	1012 (28.9)	984 (28.1)	1100 (31.4)	1131 (32.3)
Smoking status (%)					
Never	1320 (37.7)	1555 (44.4)	1702 (48.6)	1709 (48.8)	1712 (48.9)
Past	1484 (42.4)	1464 (41.8)	1422 (40.6)	1422 (40.6)	1359 (38.8)
Current	697 (19.9)	483 (13.8)	378 (10.8)	371 (10.6)	434 (12.4)
Education (%)					
<HS	410 (11.7)	333 (9.5)	287 (8.2)	298 (8.5)	277 (7.9)
HS grad	998 (28.5)	872 (24.9)	798 (22.8)	854 (24.4)	928 (26.5)
Some college	966 (27.6)	928 (26.5)	970 (27.7)	960 (27.4)	984 (28.1)
College grad	1134 (32.4)	1355 (38.7)	1443 (41.2)	1387 (39.6)	1310 (37.4)
Income (%)					
Refused	403 (11.5)	417 (11.9)	417 (11.9)	385 (11.0)	417 (11.9)
<$20,000	606 (17.3)	546 (15.6)	483 (13.8)	525 (15.0)	483 (13.8)
$20,000-$34,000	854 (24.4)	812 (23.2)	802 (22.9)	883 (25.2)	833 (23.8)
$35,000-$74,000	1075 (30.7)	1124 (32.1)	1124 (32.1)	1135 (32.4)	1124 (32.1)
≥$75,000	557 (15.9)	606 (17.3)	665 (19.0)	623 (17.8)	644 (18.4)
Depressive Symptoms[2]	340 (9.7)	305 (8.7)	312 (8.9)	291 (8.3)	392 (11.2)
History of CAD [3]	382 (10.9)	371 (10.6)	333 (9.5)	326 (9.3)	347 (9.9)
Energy intake, kcal (SD)	2096 (756)	1464 (569)	1517 (630)	1662 (657)	1609 (571)
Dietary fiber (g/d)	16.9 (8.6)	14.2 (7.7)	15.6 (8.2)	16.3 (8.5)	17.1 (8.9)
Omega-3 fats (g/d)	2.0 (1.0)	1.4 (0.7)	1.4 (0.8)	1.5 (0.8)	1.7 (0.9)
Sodium (mg/d)	2854 (1170)	1972 (854)	1989 (940)	2190 (988)	2385 (1094)
Potassium (mg/d)	2766 (1107)	2291 (980)	2543 (1012)	2699 (1058)	2926 (1152)
Vitamin C (mg/d)	86.9 (59.6)	91.6 (56.6)	120 (66.6)	121 (79.0)	116 (82.2)

Vitamin E (a-TE/d)	11.3 (5.6)	8.4 (4.4)	8.8 (4.6)	9.4 (8.4)	10.1 (5.7)
Beta-carotene (µg/d)	3412 (2838)	3160 (2582)	3579 (2978)	3706 (3194)	3814 (3368)
Folate (µg/d)	362 (167)	295 (126)	323 (148)	347 (152)	379 (166)
% energy from sweets	17.3 (10.7)	14.2 (9.5)	13.0 (8.7)	14.5 (9.2)	16.1 (10.1)
Whole grains (srv/d)	1.8 (1.6)	1.5 (1.3)	1.5 (1.3)	1.6 (1.3)	1.7 (1.4)
# Alc drinks per week	2.9 (9.1)	2.5 (6.5)	2.4 (5.4)	2.2 (5.6)	1.8 (5.6)

[1] Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes; REGARDS, Reasons for Geographic and Racial Differences in Stroke; data analyzed using ANOVA and chi-square tests for continuous and categorical variables, respectively and all comparisons statistically significant at $p<0.001$,

[2] Score ≥ 4 on Center for Epidemiological Studies-Depression-4-item

[3] CAD, coronary artery disease, defined as self-reported history of cardiovascular disease or ECG-detected at enrollment

Table 6.2. Mean differences and 95% confidence interval (CI) for average composite cognitive function score by quintile of energy-adjusted flavonoid intake for 17,509 participants in the REGARDS study[1,2]

	Q1	Q2	Q3	Q4	Q5	*P – trend*[4]
Total flavonoid I, mg/d[3]	46 (≤ 72)	92 (73-113)	141 (114-182)	251 (183-352)	599 (≥ 353)	
Model I[5]	1.00	0.06 (0.02, 0.10)	0.09 (0.05, 0.13)	0.11 (0.07, 0.14)	0.13 (0.10, 0.17)	*<0.001*
Model II[6]	1.00	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)	0.03 (-0.01, 0.06)	0.03 (-0.01, 0.06)	*0.033*
Anthocyanidins, mg/d	3.4 (≤ 4.9)	6.3 (5.0-7.8)	9.6 (7.9-11.8)	14.6 (11.9-18.5)	25.3 (≥ 18.6)	
Model I	1.00	0.05 (0.01, 0.08)	0.10 (0.06, 0.14)	0.15 (0.11, 0.19)	0.24 (0.20, 0.28)	*<0.001*
Model II	1.00	0.02 (-0.01, 0.09)	0.03 (-0.01, 0.08)	0.03 (-0.01, 0.07)	0.05 (0.02, 0.09)	*0.004*
Flavan-3-ols, mg/d	9.9 (≤ 15.2)	23.1 (15.3-35.0)	55.2 (35.1–115.6)	196.1 (115.6–269.1)	223.0 (≥ 269.2)	
Model I	1.00	0.01 (-0.02, 0.04)	0.05 (0.02, 0.07)	0.06 (0.04, 0.09)	0.14 (0.11, 0.17)	*<0.001*
Model II	1.00	0.01 (-0.03, 0.03)	0.02 (-0.01, 0.05)	0.01 (-0.01, 0.04)	0.05 (0.02, 0.08)	*0.040*
Flavanones, mg/d	2.1 (≤ 3.9)	6.3 (4.0-9.9)	16.4 (10.0–23.7)	34.9 (23.8–47.9)	58.1 (≥ 48.0)	
Model I	1.00	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.02)	0.05 (0.01, 0.09)	0.02 (-0.02, 0.06)	*0.069*
Model II	1.00	0.01 (-0.03, 0.04)	-0.02 (-0.06, 0.02)	0.03 (-0.11, 0.10)	-0.02 (-0.13, 0.10)	*0.760*
Flavones, mg/d	0.32 (≤ 0.42)	0.53 (0.43–0.63)	0.76 (0.64–0.90)	1.12 (0.91-1.45)	2.09 (≥ 1.46)	
Model I	1.00	0.13 (0.09, 0.17)	0.16 (0.12, 0.20)	0.26 (0.23, 0.30)	0.34 (0.30, 0.38)	*<0.001*
Model II	1.00	0.07 (0.03, 0.10)	0.04 (0.01, 0.07)	0.08 (0.04, 0.12)	0.12 (0.09, 0.16)	*<0.001*
Flavonols, mg/d	6.7 (≤ 8.9)	11.3 (9.0-13.5)	15.9 (13.6-18.9)	22.6 (19.0-27.7)	35.8 (≥ 27.8)	
Model I	1.00	0.15 (0.11, 0.19)	0.22 (0.18, 0.25)	0.24 (0.20, 0.28)	0.34 (0.30, 0.38)	*<0.001*
Model II	1.00	0.06 (0.03, 0.09)	0.07 (0.03, 0.11)	0.06 (0.03, 0.10)	0.12 (0.08, 0.16)	*<0.001*
Isoflavones, mg/d	0.12 (≤ 0.18)	0.25 (0.19-0.33)	0.43 (0.34–0.56)	0.73 (0.57–1.02)	1.68 (≥ 1.03)	
Model I	1.00	0.10 (0.06, 0.13)	0.19 (0.15, 0.22)	0.27 (0.23, 0.31)	0.28 (0.24, 0.32)	*0.01*
Model II	1.00	0.01 (-0.03, 0.05)	0.02 (-0.01, 0.06)	0.05 (0.01, 0.09)	0.05 (0.01, 0.09)	*0.001*
Proanthocyanidin, mg/d	33.8 (≤ 46.8)	58.7 (46.9-70.9)	84.0 (71.0-98.8)	116.9 (98.8-141.3)	181.5 (≥ 141.4)	
Model I	1.00	0.10 (0.06, 0.14)	0.19 (0.15, 0.23)	0.24 (0.20, 0.27)	0.22 (0.19, 0.21)	*<0.001*
Model II	1.00	0.02 (-0.02, 0.05)	-0.01 (-0.04, 0.03)	0.03 (-0.01, 0.07)	0.03 (-0.01, 0.06)	*0.202*
Total Flavonoid II, mg/d[7]	108 (≤ 149)	180 (150-212)	250 (213-298)	367 (299-478)	717 (≥ 479)	
Model I	1.00	0.13 (0.09, 0.16)	0.16 (0.13, 0.21)	0.17 (0.13, 0.21)	0.20 (0.16, 0.24)	*0.47*
Model II	1.00	0.01 (-0.03, 0.03)	0.01 (-0.02, 0.05)	0.02 (-0.02, 0.05)	0.03 (-0.01, 0.06)	*0.12*

1. REasons for Geographic and Racial Disparities in Stroke study (REGARDS). Mean differences (95% CI) were calculated with the use of repeated measures models in PROC MIXED using an unstructured correlation structure. To help interpret the mean differences, we found that one year of age was associated with a mean decrease of 0.036 standard units on the composite score. For example, the mean differences that we observed for extreme categories of flavone intake were equivalent to roughly 3 years of age.
2. The composite cognitive score was calculated by standardizing (using z-scores) on all three available cognitive tests in the battery at each cognitive assessment and averaging them together.
3. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes
4. P-trend values were calculated with the use of the Wald test.
5. Model I: adjusted for age, total energy and sex
6. Model II: model adjusted for age, total energy, sex, race, region of residence, educational attainment, household income, exercise, smoking status, presence of depressive symptoms and percent energy from sweets
7. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, isoflavone and proanthocyanidin intake.

Table 6.3. Mean difference and 95% CI for average composite cognitive function score by intake of flavonoid-rich foods for 14,370 participants in the REGARDS study[1,2]

	1	2	3	4
Wine	0	≤1 servings/wk	2-4 servings/ wk	≥5 servings/ wk
Model I[3]	1.00	0.20 (0.18, 0.13)	0.25 (0.20, 0.20)	0.33 (0.29, 0.37)
Model II[4]	1.00	0.09 (0.05, 0.13)	0.05 (0.01, 0.11)	0.08 (0.03, 0.14)
Citrus fruits/juices	< 1 serving/wk	1-6 servings/ wk	≥ 7 servings/ wk	
Model I[3]	0	0.02 (-0.01, 0.05)	0.04 (0.01, 0.08)	
Model II[4]	0	0.01 (-0.01, 0.04)	0.03 (-0.01, 0.06)	
Apples and pears	< 1 serving/ wk	1-6 servings/ wk	≥ 7 servings/ wk	
Model I[3]	0	0.07 (0.04, 0.10)	0.06 (-0.01, 0.12)	
Model II[4]	0	0.01 (-0.01, 0.04)	-0.03 (-0.08, 0.02)	
Berries	< 1 serving/mo	1-3 servings/mo	≥ 4 serving/mo	
Model I[3]	0	0.15 (0.12, 0.18)	0.24 (0.18, 0.29)	
Model II[4]	0	0.03 (-0.01, 0.06)	0.09 (0.04, 0.14)	
Legumes	< 1 serving/ wk	1-6 servings/ wk	≥ 7 servings/ wk	
Model I[3]	1.00	0.02 (-0.02, 0.06)	-0.02 (-0.06, 0.02)	
Model II[4]	1.00	0.02 (-0.02, 0.05)	0.01 (-0.03, 0.04)	
100% fruit juice (other than citrus)	< 1 serving/ wk	1-6 servings/ wk	≥ 7 servings/ wk	
Model I[3]	0	0.11 (0.07, 0.14)	0.11 (-0.02, 0.05)	
Model II[4]	0	0.07 (0.04, 0.11)	0.06 (0.03, 0.09)	
Chocolate	0 servings/ wk	> 0 servings/ wk		
Model I[3]	0	0.14 (0.09, 0.19)		
Model II[4]	0	0.07 (0.04, 0.12)		
Peanuts/nuts	0 servings/ wk	> 0 servings/ wk		
Model I[3]	0	0.26 (0.20, 0.31)		
Model II[4]	0	0.10 (0.05, 0.15)		
Tea	0 servings/ wk	> 0 servings/ wk		
Model I[3]	1.00	0.14 (0.11, 0.17)		
Model II[4]	1.00	0.09 (0.04, 0.13)		
Tofu	0 servings/ wk	> 0 servings/ wk		
Model I[3]	1.00	0.23 (0.19, 0.27)		
Model II[4]	1.00	0.06 (0.01, 0.11)		

1. Mean differences (95% CI) were calculated with the use of repeated measures models in PROC MIXED. Average composite cognitive score was calculated by estimating the mean of standardized (using z-scores) cognitive tests in the battery. To help interpret the adjusted mean differences, we found that one year of age was associated with a mean

decrease of 0.036 standard units on the global score. For example, the mean differences that we observed for extreme categories of wine intake were equivalent to roughly 2 years of cognitive age difference. REasons for Geographic and Racial Disparities in Stroke study (REGARDS).

2. The composite cognitive score was calculated by standardizing (using z-scores) on all three available cognitive tests in the battery at each cognitive assessment and averaging them together.

3. Model I: adjusted for age, total energy and sex

4. Model II: model adjusted for age, total energy, sex, race, region of residence, educational attainment, household income, exercise, smoking status, presence of depressive symptoms, percent energy from sweets

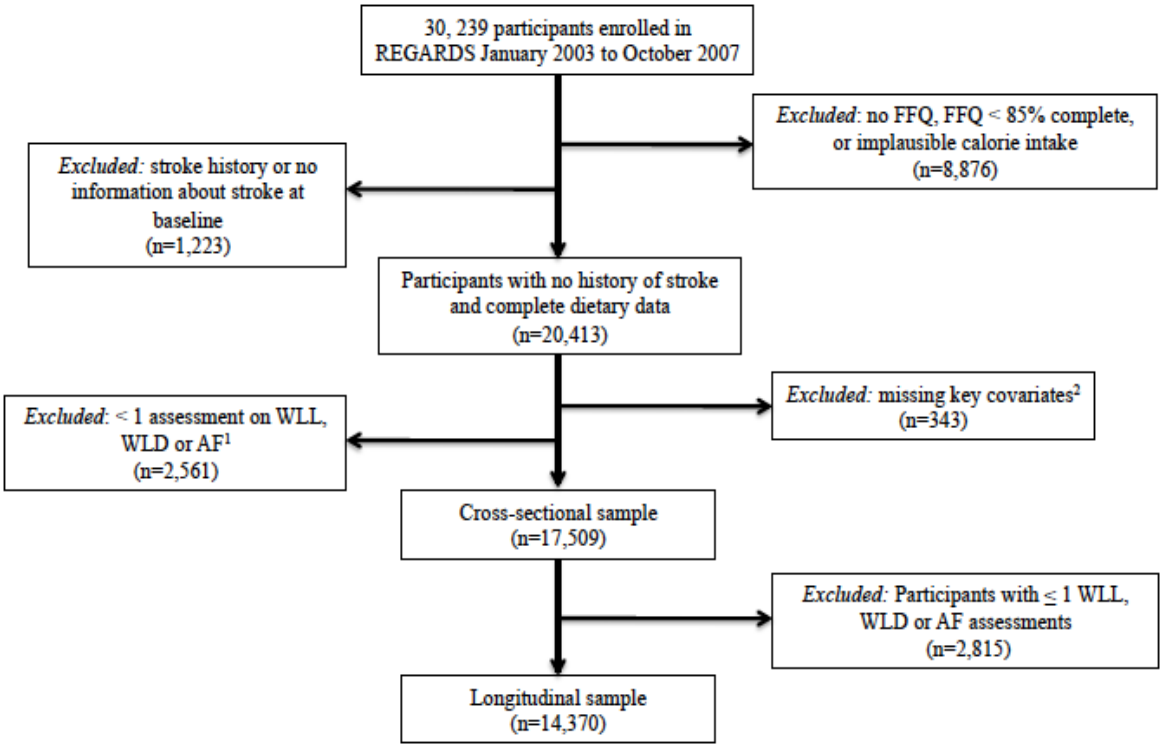
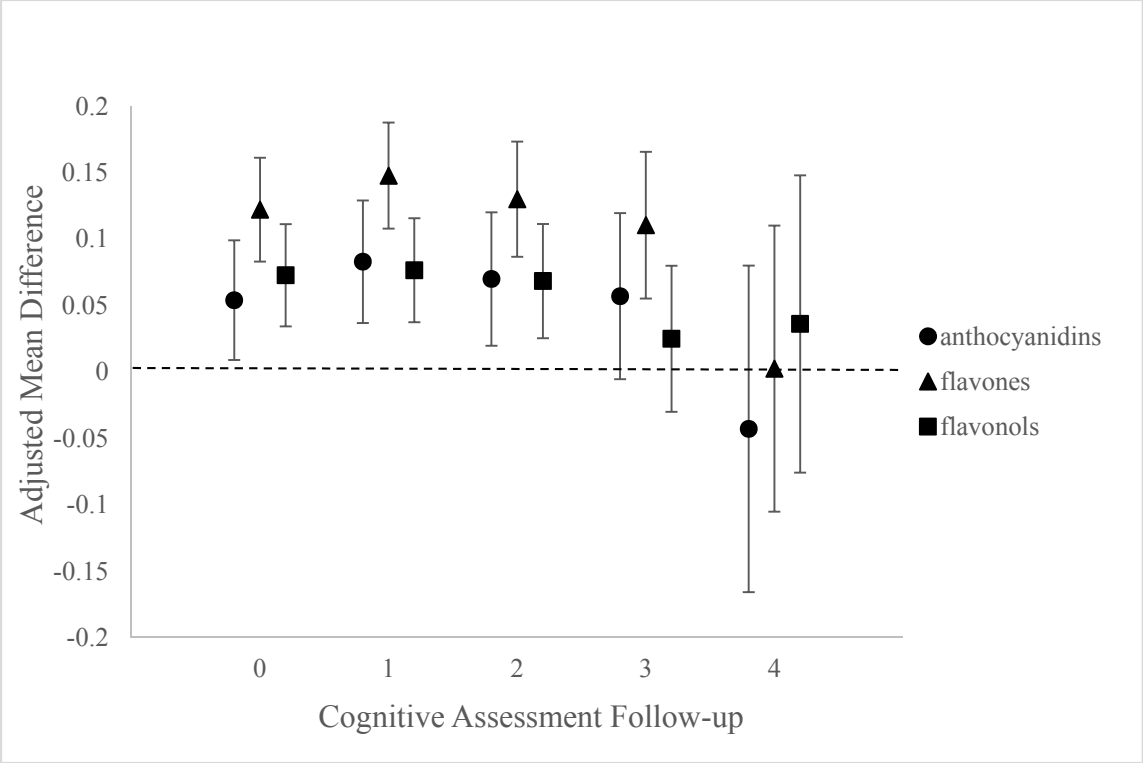


Figure 6.1. Participant flow diagram

1. AF=Animal Fluency Test; WLL=Word List Learning; WLD=Word List Recall

2. Categories for missing data are not mutually exclusive. Missing data for covariates include physical activity (n=178), smoking status (n=55), education (n=6) and depressive symptoms (n=98).



	0	1	2	3	4
N	14,186	14,308	11,176	6,971	1,460
Anthocyanin	0.05 (0.01, 0.10)	0.08 (0.04, 0.13)	0.07 (0.02, 0.12)	0.06 (-0.01, 0.12)	-0.01 (-0.17, 0.08)
Flavone	0.12 (0.08, 0.16)	0.15 (0.11, 0.19)	0.13 (0.09, 0.17)	0.11 (0.06, 0.17)	0.01 (-0.11, 0.11)
Flavonol	0.07 (0.03, 0.11)	0.08 (0.04, 0.12)	0.07 (0.03, 0.11)	0.02 (-0.03, 0.07)	0.04 (-0.08, 0.15)

Figure 6.2. Adjusted Mean Differences in Average Composite Cognitive z-score comparing extreme quintiles (Q5 versus Q1) of select flavonoid intake over time. A mean difference above 0 signifies better cognitive function in Q5 compared to Q1.

Adjusted for age, total energy intake, sex, self-reported race, region of residence, educational attainment, household income, physical activity, smoking status, presence of depressive symptoms and percent of energy consumed from sweets.

Table S.6.1. Flavonoid subclasses, compounds and common food sources included in the US Department of Agriculture flavonoid databases

USDA Database	Flavonoid subclass (common food sources)	Flavonoid Compounds
Provisional Flavonoid Addendum[1]	Anthocyanidins (blueberries, strawberries, red wine)	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin
	Flavan-3-ols (apples, tea, chocolate)	Catechin, Epicatechin, Epicatechin 3-gallate, Epigallocatechin, Epigallocatechin 3-gallate, Gallocatechin, Theaflavin, Theaflavin 3-gallate, Theaflavin 3'-gallate, Theaflavin 3,3'-digallate, Thearubigins
	Flavanones (citrus fruit and juices)	Eriodictyol, Hesperetin, Naringenin
	Flavones (celery, parsley, green pepper)	Apigenin, Luteolin
	Flavonols (onion, apple, broccoli)	Isorhamnetin, Kaempferol, Myricetin, Quercetin
	Isoflavones (tofu, soymilk, soy-based foods)	Daidzein, Genistein, Glycitein
Proanthocyanidin[2]	Proanthocyanidins (apples, chocolate, red wine)	Monomers, dimers, trimers, 4-6mers, 7-10mers, polymers

1. U.S. Department of Agriculture Agricultural Research Service. USDA database for the proanthocyanidin content of selected foods. Beltsville, MD: Agricultural Research Service Nutrient Data Laboratory, 2004.

2. Sebastian RS, Goldman JD, Martin CL, Steinfeldt LC, Enns CW, Moshfegh AJ. Flavonoid Values for USDA Survey Foods and Beverages 2007-2008: Provisional Flavonoid Addendum to the USDA Food and Nutrient Database for Dietary Studies,4.1, and Flavonoid Intake Files from What We Eat in America (WWEIA), National Health and Nutrition Examination Survey (NHANES) 2007-2008. In: U.S. Department of Agriculture ARS, Food Surveys Research Group., ed. Beltsville, MD, 2014.

Table S.6.2. Proportional contribution of Block98 FFQ items to total flavonoid and flavonoid subclass intake in the REasons for Geographic and Racial Differences in Stroke (REGARDS) Study, 2003-2007.

Total Flavonoid I[1]		Total Flavonoid II[2]	
Food source	%	Food source	%
Tea[3]	79	Tea[3]	63
Orange juice	9	Apple or pears	8
Orange	3	Cake[7]	6
Wine[4]	2	Orange juice	4
100% real fruit juice, not orange	2	Pies and cobbler[8]	4
Apples or pears, raw	2	Canned fruit[6]	4
Other vegetables[5]	2	Wine[4]	4
Salad greens	<1	100% real fruit juice, not orange	2
Canned fruit[6]	<1	Peanuts and nuts	1
Grapefruit, raw	<1	Beans	1
Anthocyanidins		Flavan-3-ols	
Food source	%	Food source	%
Wine[4]	27	Tea[3]	94
100% real fruit juice, not orange	16	Apples or pears	1
Berries	14	100% real fruit juice, not orange	1
Cole slaw	8	Bananas	1
Vegetable stews[9]	8	Wine[4]	1
Yogurt with fruit	7	Canned fruit[6]	1
Other fruits[8]	6		
Canned fruit[6]	4		
Other vegetables[5]	4		
Apples or pears	3		
Flavanone		Flavone	
Food source	%	Food source	%
Orange juice	67	Other vegetables[5]	53
Oranges	21	Salad greens	14
Grapefruit	7	Tea[3]	7
100% real fruit juice, not orange	2	Spinach	5
Liquor/cocktails	1	Cantaloupe	4
Tomato	<1	Orange	3
		Vegetable stews[9]	3
		Apple or pear	2
		Broccoli	2
		Grapefruit	1

Flavonol		Isoflavones	
Food source	%	Food source	%
Tea[3]	44	Soymilk	48
Salad greens	18	Peanuts and nuts	18
Other vegetables[5]	12	Tofu	17
Dark leafy greens	8	Other vegetables[5]	6
Apple or pears	6	Meat substitutes	3
Broccoli	4	Protein bars	2
100% real fruit juice, not orange	3	Bean soups	2
Wine[4]	3	Protein shakes	1
Proanthocyanidins			
Food source	%		
Apples or pears	18		
Cake[7]	16		
Pies and cobbler[8]	10		
Tea[3]	9		
Canned fruit[6]	8		
Beans	6		
Peanuts and nuts	6		
Wine[4]	6		
100% real fruit juice, not orange	4		
Berries	3		

1. Sum of flavonoid subclasses in the USDA Provisional Flavonoid Addendum (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, isoflavones)

2. Sum total flavonoid I and proanthocyanidins

3. In the Provisional Flavonoid Addendum, flavonoid values for tea are a composite consisting of 84% black tea and 16% green tea, based on published market share data.

4. A composite of red wine, white wine, cooking wine and sangria, weighted by National Health and Nutrition Examination Survey (NHANES) population-based reported intakes

5. Includes pumpkin, squash, peppers, onion, artichokes, asparagus, lima beans, bean sprouts, beets, brussel sprouts, cauliflower, celery, eggplant, mushrooms, okra, and parsnips that are cooked from fresh or frozen. Anthocyanidins primarily from eggplant; Flavones from pumpkins, squash, peppers, celery, okra; Flavonols from asparagus, peppers, brussel sprouts, okra, onion; Isoflavones from soybeans, bean sprouts

6. Canned fruit includes apricots, cherries, plums, peaches, pears, fruit cocktail and applesauce
7. Includes fruit, chocolate, and spice cakes; important sources of flavonoids include fruits, chocolate, and cinnamon.
8. Includes fruit cobblers, chocolate pies, and turnovers; important sources of flavonoids include fruits, chocolate and cinnamon
9. Mix of vegetables, with or without meat, prepared as a stew, excludes soups. Anthocyanidins primarily from eggplant; Flavones primarily from squashes, peppers, herbs
10. Anthocyanidins primarily from cherries, plums, red grapes, fruit salad

Table S.6.3. Adjusted mean differences in composite cognitive function score comparing extreme quintiles of flavonoid intake over five cognitive assessment occasions in 14,370 participants in the REGARDS study.[1,2]

	CERAD Cognitive Assessment				
	Time 1 *n*=14,186	Time 2 *n*=14,308	Time 3 *n*=11,176	Time 4 *n*=6,971	Time 5 *n*=1,460
Total flavonoid I[3]					
Model I[4]	0.12 (0.08, 0.17)	0.12 (0.07, 0.16)	0.07 (0.02, 0.13)	0.10 (-0.01, 0.21)	0.15 (0.11, 0.19)
Model II[5]	0.04 (0.01, 0.08)	0.02 (-0.01, 0.06)	0.02 (-0.02, 0.06)	-0.03 (-0.08, 0.03)	0.01 (-0.09, 0.12)
Anthocyanidin					
Model I[4]	0.25 (0.21, 0.29)	0.26 (0.22, 0.30)	0.23 (0.19, 0.28)	0.20 (0.15, 0.26)	0.16 (0.06, 0.27)
Model II[5]	0.05 (0.01, 0.10)	0.08 (0.04, 0.13)	0.07 (0.02, 0.12)	0.06 (-0.01, 0.12)	-0.04 (-0.17, 0.08)
Flavan-3-ols					
Model I[4]	0.10 (0.06, 0.15)	0.09 (0.04, 0.13)	0.07 (0.03, 0.12)	0.03 (-0.02, 0.09)	0.13 (0.02, 0.23)
Model II[5]	0.04 (0.01, 0.08)	0.02 (-0.02, 0.06)	0.01 (-0.03, 0.06)	-0.03 (-0.08, 0.02)	0.08 (-0.02, 0.18)
Flavanone					
Model I[4]	0.04 (-0.01, 0.08)	0.03 (-0.01, 0.08)	0.01 (-0.03, 0.06)	-0.01 (-0.07, 0.04)	0.01 (-0.10, 0.12)
Model II[5]	0.01 (-0.02, 0.05)	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.04)	-0.04 (-0.10, 0.01)	-0.02 (-0.12, 0.10)
Flavone					
Model I[4]	0.33 (0.29, 0.38)	0.35 (0.31, 0.39)	0.34 (0.29, 0.38)	0.3121 (0.26, 0.37)	0.20 (0.09, 0.31)
Model II[5]	0.12 (0.08, 0.16)	0.15 (0.11, 0.19)	0.13 (0.09, 0.17)	0.11 (0.06, 0.17)	0.01 (-0.11, 0.11)
Flavonol					
Model I[4]	0.27 (0.23, 0.31)	0.28 (0.23, 0.32)	0.27 (0.22, 0.31)	0.23 (0.17, 0.28)	0.19 (0.09, 0.29)
Model II[5]	0.07 (0.03, 0.11)	0.08 (0.04, 0.12)	0.07 (0.03, 0.11)	0.02 (-0.03, 0.07)	0.04 (-0.08, 0.15)
Isoflavone					
Model I[4]	0.21 (0.17, 0.25)	0.22 (0.17, 0.26)	0.18 (0.14, 0.23)	0.18 (0.12, 0.23)	0.10 (-0.01, 0.21)
Model II[5]	0.06 (0.02, 0.10)	0.07 (0.03, 0.11)	0.04 (-0.01, 0.08)	0.03 (-0.02, 0.09)	-0.03 (-0.01, 0.07)
Proanthocyanidin					
Model I[4]	0.17 (0.13, 0.21)	0.14 (0.10, 0.18)	0.14 (0.10, 0.19)	0.11 (0.05, 0.16)	0.10 (-0.01, 0.21)

Model II[5]	0.05 (0.01, 0.09)	0.02 (-0.02, 0.06)	0.02 (-0.02, 0.06)	-0.02 (-0.07, 0.04)	-0.02 (-0.12, 0.09)
Total Flavonoid II[6]					
Model I[4]	0.15 (0.11, 0.19)	0.13 (0.09, 0.17)	0.12 (0.08, 0.17)	0.07 (0.02, 0.13)	0.12 (0.01, 0.23)
Model II[5]	0.04 (0.01, 0.08)	0.03 (-0.01, 0.07)	0.02 (-0.03, 0.06)	-0.03 (-0.09, 0.02)	0.04 (-0.06, 0.14)

1. REasons for Geographic and Racial Disparities in Stroke study (REGARDS). Mean differences (95% CI) were calculated with the use of repeated measures models in PROC MIXED using an unstructured correlation structure. To help interpret the mean differences, we found that one year of age was associated with a mean decrease of 0.036 standard units on the composite score. For example, the mean differences that we observed for extreme categories of flavone intake were equivalent to roughly 3 years of age.

2. The composite cognitive score was calculated by standardizing (using z-scores) on all three available cognitive tests in the battery at each cognitive assessment and averaging them together.

3. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, and isoflavone intakes

4. Model I: adjusted for age, total energy and sex

5. Model II: model adjusted for age, total energy, sex, race, region of residence, educational attainment, household income, exercise, smoking status, presence of depressive symptoms and percent energy from sweets

6. Sum of anthocyanidin, flavan3-ol, flavanone, flavone, flavonol, isoflavone and proanthocyanidin intake.

Table S.6.4. Adjusted mean differences in composite cognitive function score comparing extreme intake of flavonoid-rich food over five cognitive assessment occasions in 14,370 participants in the REGARDS study.[1,2]

	CERAD Cognitive Assessment				
	Time 1 *n*=14,186	Time 2 *n*=14,308	Time 3 *n*=11,176	Time 4 *n*=6,971	Time 5 *n*=1,460
Wine					
Model I[3]	0.33 (0.29, 0.37)	0.36 (0.32, 0.49)	0.35 (0.30, 0.40)	0.36 (0.31, 0.42)	0.18 (0.08, 0.28)
Model II[4]	0.08 (0.04, 0.13)	0.11 (0.07, 0.15)	0.10 (0.06, 0.15)	0.11 (0.06, 0.17)	-0.05 (-0.16, 0.04))
Citrus fruits/juice					
Model I[3]	0.04 (0.01, 0.08)	0.03 (-0.01, 0.06)	0.02 (-0.01, 0.06)	0.02 (-0.02, 0.07)	0.02 (-0.07, 0.11)
Model II[4]	0.03 (-0.01, 0.06)	0.02 (-0.02, 0.05)	0.02 (-0.02, 0.05)	0.01 (-0.04, 0.05)	0.02 (-0.07, 0.11)
Apples and pears					
Model I[3]	0.06 (-0.01, 0.12)	0.10 (0.04, 0.16)	0.07 (0.01, 0.14)	0.10 (0.02, 0.18)	0.28 (0.15, 0.41)
Model II[4]	-0.03 (-0.08, 0.02)	0.01 (-0.04, 0.07)	-0.01 (-0.08, 0.05)	0.01 (-0.06, 0.09)	0.17 (0.04, 0.30)
Berries					
Model I[3]	0.24 (0.18, 0.29)	0.26 (0.20, 0.31)	0.22 (0.16, 0.28)	0.27 (0.19, 0.34)	0.07 (-0.05, 0.19)
Model II[4]	0.09 (0.04, 0.14)	0.10 (0.05, 0.16)	0.07 (0.02, 0.13)	0.12 (0.05, 0.20)	-0.09 (-0.22, 0.04)
Legumes					
Model I[3]	0.04 (-0.01, 0.08)	0.02 (-0.02, 0.06)	-0.02 (-0.07, 0.03)	-0.02 (-0.08, 0.03)	0.08 (-0.03, 0.19)
Model II[4]	-0.01 (-0.04, 0.03)	-0.04 (-0.08, 0.01)	-0.04 (-0.09, 0.01)	0.07 (-0.04, 0.18)	0.02 (-0.02, 0.06)
100% real fruit juice					
Model I[3]	0.01(-0.02, 0.05)	0.01 (-0.02, 0.05)	0.03 (-0.01, 0.07)	0.02 (-0.02, 0.07)	-0.03 (-0.13, 0.06)
Model II[4]	0.06 (0.03, 0.10)	0.07 (0.03, 0.10)	0.09 (0.05, 0.12)	0.07 (0.02, 0.12)	0.02 (-0.07, 0.12)
Chocolate					
Model I[3]	0.14 (0.10, 0.19)	0.19 (0.14, 0.23)	0.19 (0.14, 0.24)	0.18 (0.12, 0.25)	-0.01 (-0.14, 0.12)
Model II[4]	0.08 (0.04, 0.12)	0.13 (0.09, 0.17)	0.13 (0.08, 0.18)	0.12 (0.06, 0.19)	-0.05 (-0.18, 0.08)
Peanuts and nuts					
Model I[3]	0.26 (0.21, 0.31)	0.30 (0.25, 0.35)	0.32 (0.26, 0.38)	0.23 (0.16, 0.30)	0.14 (-0.02, 0.29)

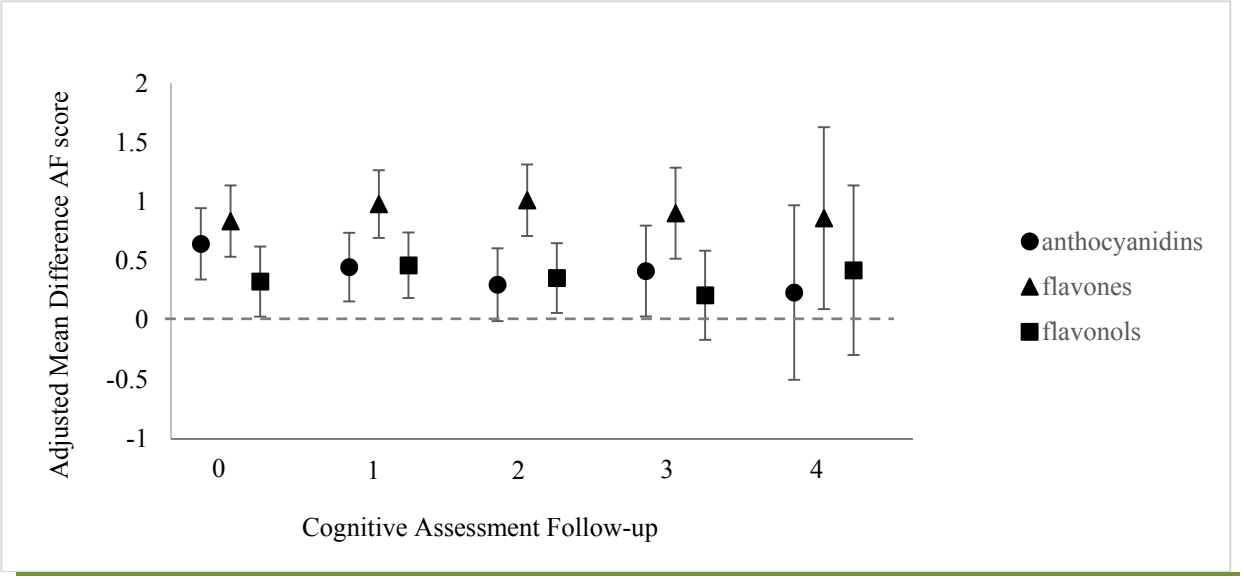
Model II[4]	0.10 (0.05, 0.14)	0.14 (0.09, 0.19)	0.16 (0.10, 0.21)	0.07 (0.01, 0.14)	-0.01 (-0.15, 0.14)
Tea					
Model I[3]	0.14 (0.11, 0.18)	0.12 (0.08, 0.15)	0.14 (0.10, 0.18)	0.11 (0.07, 0.16)	0.08 (-0.01, 0.18)
Model II[4]	0.09 (0.05, 0.12)	0.06 (0.03, 0.10)	0.09 (0.06, 0.13)	0.06 (0.01, 0.10)	0.06 (-0.03, 0.15)
Tofu					
Model I[3]	0.24 (0.20, 0.27)	0.28 (0.25, 0.32)	0.27 (0.23, 0.31)	0.27 (0.22, 0.32)	0.22 (0.11, 0.33)
Model II[4]	0.06 (0.02, 0.10)	0.11 (0.07, 0.15)	0.09 (0.05, 0.13)	0.09 (0.04, 0.14)	0.04 (-0.07, 0.15)

1. REasons for Geographic and Racial Disparities in Stroke study (REGARDS). Mean differences (95% CI) were calculated with the use of repeated measures models in PROC MIXED using an unstructured correlation structure. To help interpret the mean differences, we found that one year of age was associated with a mean decrease of 0.036 standard units on the composite score. For example, the mean differences that we observed for extreme categories of flavone intake were equivalent to roughly 3 years of age.

2. The composite cognitive score was calculated by standardizing (using z-scores) on all three available cognitive tests in the battery at each cognitive assessment and averaging them together.

3. Model I: adjusted for age, total energy and sex

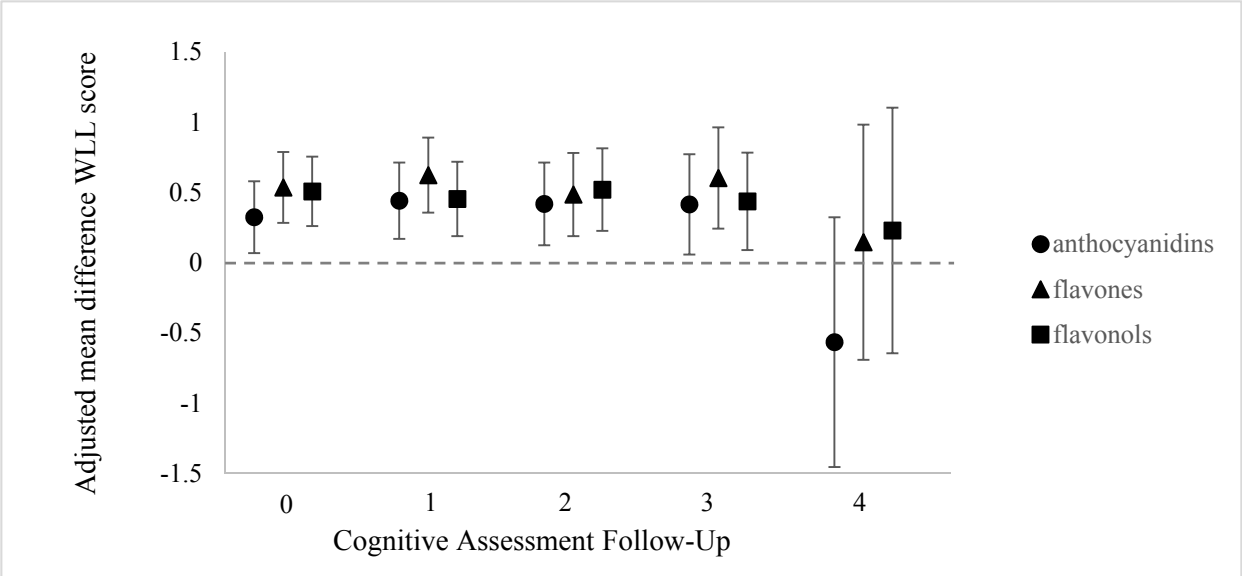
4. Model II: model adjusted for age, total energy, sex, race, region of residence, educational attainment, household income, exercise, smoking status, presence of depressive symptoms and percent energy from sweet



	0	1	2	3	4
N	11,278	13,007	9,957	5,247	1,020
Anthocyanidin	0.64 (0.34, 0.95)	0.45 (0.16, 0.74)	0.30 (-0.01, 0.61)	0.41 (0.03, 0.80)	0.23 (-0.50, 0.97)
Flavone	0.84 (0.53, 1.13)	0.98 (0.69, 1.26)	1.01 (0.70, 1.31)	0.90 (0.51, 1.29)	0.86 (0.09, 1.63)
Flavonol	0.33 (0.03, 0.62)	0.46 (0.19, 0.74)	0.36 (0.06, 0.65)	0.21 (-0.17, 0.56)	0.42 (-0.29, 1.13)

Figure S.6.1A Adjusted Mean Differences in Animal Fluency test raw score comparing extreme quintiles (Q5 versus Q1) of select flavonoid intake over time. A mean difference above 0 signifies better cognitive function in Q5 compared to Q1.

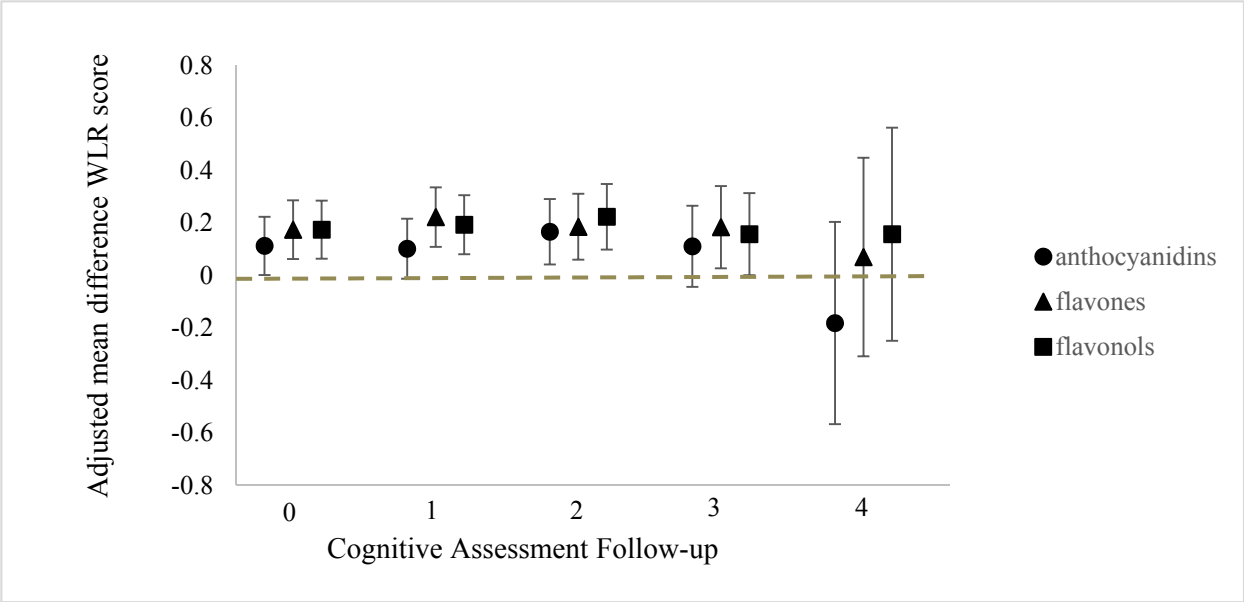
Adjusted for age, energy intake, sex, self-reported race, region of residence, educational attainment, household income, physical activity, smoking status, presence of depressive symptoms and percent of calories consumed from sweets.



	0	1	2	3	4
N	12,501	10,751	8,141	4,791	674
Anthocyanidin	0.32 (0.07, 0.58)	0.44 (0.17, 0.71)	0.42 (0.12, 0.71)	0.42 (0.06, 0.77)	-0.57 (-1.45, 0.32)
Flavone	0.54 (0.28, 0.78)	0.62 (0.36, 0.89)	0.49 (0.19, 0.78)	0.60 (0.24, 0.96)	0.15 (-0.70, 0.98)
Flavonol	0.51 (0.26, 0.75)	0.45 (0.19, 0.72)	0.52 (0.23, 0.81)	0.44 (0.09, 0.78)	0.23 (-0.65, 1.10)

Figure S.6.1B Adjusted Mean Differences in Word List Learning raw score comparing extreme quintiles (Q5 versus Q1) of select flavonoid intake over time. A mean difference above 0 signifies better cognitive function in Q5 compared to Q1.

Adjusted for age, energy intake, sex, self-reported race, region of residence, educational attainment, household income, physical activity, smoking status, presence of depressive symptoms and percent of energy consumed from sweets.



	0	1	2	3	4
N	12,150	10,619	8,053	4,734	666
Anthocyanidin	0.11 (0.01, 0.22)	0.10 (-0.01, 0.22)	0.17 (0.04, 0.29)	0.11 (-0.04, 0.26)	-0.18 (-0.57, 0.20)
Flavone	0.17 (0.06, 0.29)	0.22 (0.11, 0.34)	0.19 (0.06, 0.31)	0.18 (0.03, 0.34)	0.07 (-0.31, 0.45)
Flavonol	0.17 (0.06, 0.28)	0.19 (0.08, 0.31)	0.22 (0.10, 0.35)	0.16 (0.01, 0.31)	0.16 (-0.24, 0.56)

Figure S.6.1C Adjusted Mean Differences in Word List Recall raw score comparing extreme quintiles (Q5 versus Q1) of select flavonoid intake over time. A mean difference above 0 signifies better cognitive function in Q5 compared to Q1.

Adjusted for age, energy intake, sex, self-reported race, region of residence, educational attainment, household income, physical activity, smoking status, presence of depressive symptoms and percent of energy consumed from sweets.

Chapter 7: Summary and Future Directions

Summary

Flavonoids are bioactive, polyphenolic compounds found ubiquitously in vascular plants and have been associated with better cardiovascular and cognitive health. Overwhelmingly, studies examining health effects of flavonoids have been conducted in racially and geographically limited populations. Furthermore, a lack of comprehensive dietary flavonoid composition tables has limited previous research. The goal of this dissertation was to investigate associations of flavonoid intake with incident stroke and acute coronary heart disease as well as with cognitive function, in a biracial, national cohort using a recently expanded flavonoid database provided by the U.S. Department of Agriculture.

In the first dissertation project, we used data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study to examine the association of reported dietary total flavonoid and flavonoid subclass intakes with incident ischemic stroke. Our results suggest that flavanone, a subclass of flavonoids consumed largely from citrus fruits, was associated with a 27 % reduction in relative risk of first ischemic stroke. There were no significant associations for other dietary flavonoid subclasses and stroke. Results suggest that specific flavonoid subclasses are more important for stroke incidence than the total of all flavonoids consumed. These findings were consistent with previous studies of flavonoid intake and incident stroke in predominantly white and geographically limited cohorts, thereby extending those findings to a much larger portion of the U.S. population. Several potential mechanisms of action may mediate the inverse association

observed between flavanone intake and incident stroke. These include improved vascular function, such as lowering blood pressure and improving flow-mediated dilation, as well as the ability of flavanones to cross the blood brain barrier and inhibit inflammatory signaling.

In the second dissertation project, we used data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study to study the association of reported dietary total flavonoid and flavonoid subclass intakes with incident acute coronary heart disease events (CHD). Our results suggest that anthocyanidins and proanthocyanidins were associated with a 35% and 38% reduction in relative risk of incident acute CHD for anthocyanidins and proanthocyanidins, respectively. There were no significant associations for other flavonoid intake variables and CHD events. Again, flavonoid subclass intakes appeared to be more relevant than total flavonoid intake. The association between anthocyanidin intake and CHD was consistent with previous studies of flavonoid intake and incident CHD in a predominantly white and geographically limited cohort, thereby extending those findings to a much larger segment portion of the U.S. population. The association between proanthocyanidin intake and acute CHD were new findings. Several potential mechanisms could explain the beneficial association between anthocyanidin intake and incident CHD. Anthocyanidins reduce inflammatory activity in the vascular endothelium, improve vascular reactivity and may also improve glycemia. Compounds in the proanthocyanidin class are less well understood than other flavonoid subclasses, however, proanthocyanidins may improve lipid metabolism, improve satiety signaling as well as protect against thrombosis and elevated blood pressure.

In the third dissertation project, we used data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study to study the association of reported dietary total flavonoid and flavonoid subclass intakes with cognitive function over time. Our results suggest that anthocyanidins, flavones and flavonols were significantly associated with better cognitive test scores over roughly 6 to 8 years of follow-up. The magnitude of the effect was comparable to roughly 2-3 years of age. Though this is a relatively mild effect at the individual level, on a population level, a small delay in onset of cognitive decline may have substantial public health benefits. Flavonoid subclass intakes appeared to be more relevant than total flavonoid intake. Similar to flavanones, the ability to cross the blood-brain barrier has been described for anthocyanidins, flavones and flavonols. Once across the blood-brain barrier, these flavonoid subclasses likely exert a variety of anti-inflammatory actions as well as support long-term potentiation and neurogenesis. The findings for anthocyanidin, flavone and flavonol intakes were consistent with previous studies of flavonoid intake and cognitive function in European and U.S. cohorts.

We also found that flavonoid intake often differed by race and region of residence. Black participants consumed fewer flavonoids in total and fewer of each subclass of flavonoids, except for flavanones. This was consistent with a recent NHANES examination of flavonoid consumption in the U.S. using a flavonoid database similar to ours. Regional differences in flavonoid intake were more complex. Those living in the stroke belt were more likely to consume flavan-3-ols and less likely to consume anthocyanidins, flavanones, and isoflavones than those living outside of the stroke belt.

Results from these three dissertation projects extend findings from previous studies that were limited in racial and geographic diversity. The differences in flavonoid intake by race and region of residence may help to explain some of the racial and regional disparities in outcomes that have been observed in the literature, though not racial disparities in stroke. However, it is impractical to make suggestions about specific flavonoid subclasses given the complexity of the flavonoid content of foods. It is perhaps more practical to emphasize the importance of consuming plant-based foods, especially fruits and vegetables, of a variety of colors on a daily basis.

Future Directions

Project 1: **Is increased flavonoid intake associated with non-traditional stroke risk factors, such as self-reported stroke symptoms, atrial fibrillation, retinal vascular disease and carotid intima-media thickness?**

Self-reported stroke symptoms (sudden onset of weakness, numbness, blindness, difficulty in communicating and difficulty understanding) are associated with Framingham Stroke Risk Score[295], increased relative risk of cognitive impairment[205] and lower quality of life.[296] Self-report of any stroke symptom has been associated with a 36% increased relative risk of future stroke, increasing to 46% and 77% greater relative risk of stroke for those reporting two or three stroke symptoms, respectively. By comparison, the prediction of stroke occurrence was stronger for stroke symptoms than prevalent diabetes and heart disease.[297] While these stroke symptoms may represent undiagnosed prevalent cases of TIA or stroke, they may also be related to other neurologic conditions such as migraine, seizures, dementia, and non-vascular occlusive

ocular disease. Those who report stroke symptoms in the absence of diagnosed stroke or TIA represent a high-risk population for stroke and cognitive impairment. If flavonoid intake (especially flavanone, anthocyanidin, flavone and flavonol intake) were associated with self-reported stroke symptoms, this finding would support a causal association between flavonoid intake, stroke and cognitive function. **Hypothesis**: Greater reported intakes of select flavonoid subclasses, anthocyanidins, flavanones, flavones and flavonols, are associated with lower relative risk of self-reported stroke symptoms in those without a history of stroke or TIA.

Atrial fibrillation is a common arrhythmia and a strong risk factor for stroke and other cardiovascular outcomes.[298] There are likely many pathophysiologic factors underlying the development of atrial fibrillation, including inflammation, oxidative stress, and increased autonomic nervous system activity that may facilitate induction of atrial fibrillation.[299] Very few studies have been published examining a potential role of flavonoids or flavonoid-rich foods in relation to atrial fibrillation, though the anti-inflammatory and indirect antioxidant properties of flavonoids are biologically plausible pathways by which atrial fibrillation could be reduced.[300] The phenomenon of "holiday heart," in which otherwise healthy people develop cardiac arrhythmias after binge drinking, raises the question whether flavonoids, which are rich in wine, could contribute to arrhythmia. In two different surveys of patients with paroxysmal atrial fibrillation, patients commonly identified wine, chocolate, onions, and nuts, all flavonoid-rich foods, as triggers.[301,302] The response to triggering foods may be mediated by excess adrenergic tone. Nearly half of all dopamine synthesized in humans is formed in the intestines and roughly 97% is inactivated by sulfotransferase (SULT) enzymes before it can enter the

bloodstream. A variety of polyphenols, including the flavanones, flavones, and flavonols, are potent inhibitors of SULT enzymes, leading to unusually high concentrations of dopamine entering systemic circulation.[303] Similar biochemical pathways likely mediate migraine headaches triggered by these same foods. Though greater flavonoid intake may be beneficial for cardiovascular health, it is possible that in some cases flavonoids may trigger arrhythmias that increase the risk of stroke and other cardiovascular events. **Hypothesis**: Greater consumption of flavonoid subclasses commonly found in food triggers, such as proanthocyanidins, anthocyanidins and flavonols, is associated with greater prevalence of atrial fibrillation.

The retinal vasculature shares similar anatomic and physiological characteristics with cerebral vessels. The retina is an extension of the central nervous system, with a retinal blood barrier similar to the blood brain barrier. Abnormalities identified in retinal microvasculature likely reflect similar changes in cerebral vessels[304] and retinal vascular abnormalities are also strongly associated with long-term stroke risk.[305] An important and unique feature of the retinal vasculature is that it can be noninvasively visualized *in vivo*. Not only are retinal abnormalities such as hypertensive retinopathy, diabetic retinopathy, narrowing of the retinal artery and dilatation of the retinal vein, associated with increased risk of incident stroke or stroke mortality, these conditions are also associated with blindness.[305] An examination of flavonoid intake and retinal health may provide insights into the role of flavonoids in cerebrovascular health. Moreover, flavonoid intake may be associated with retinal health as well, providing a potential modifiable risk factor to prevent or delay vision loss. Several U.S. epidemiologic cohorts, have collected data

about diet and retinal health and could serve as data sources to examine these associations, including the National Health and Nutrition Examination Study (NHANES), the Atherosclerosis and Risk in Communities Study (ARIC), the Cardiovascular Health Study (CHS), and the Beaver Dam Eye Study. **Hypothesis**: Greater intake of anthocyanidins, which have been favorably associated with a variety of endothelial function measures, is associated with better retinal and retinal microvascular health, as measured by retinal artery and retinal vein diameter, presence of diabetic retinopathy and presence of hypertensive retinopathy.

Carotid intima media thickness (cIMT) is positively associated with risk of stroke and coronary heart disease.[306,307] Specific properties of carotid plaques are also associated with atherosclerotic plaque irregularities in other vascular beds.[308-311] Traditionally, carotid ultrasound has been the modality of choice to assess cIMT. Newer imaging modalities, such as MRI, allow for measurement of cIMT as well as characterization of the carotid plaques, to include detection of a necrotic core, calcification and intraplaque hemorrhage. Intraplaque hemorrhage may be particularly relevant for stroke risk.[306,312] In the only population-based study of flavonoid subclass intake and cIMT, as measured by ultrasound, there was an inverse, cross-sectional association between flavan-3-ol intake and cIMT among Finnish male smokers.[313] A prospective analysis is warranted to assess temporality, assess changes in cIMT over time and also include a more demographically diverse cohort. Evaluation of the association btween flavonoid subclass intake and carotid plaque composition, such as the presence of intraplaque hemorrhage, which is particularly associated with stroke, could clarify the role of flavanone intake in incident

ischemic stroke. **Hypothesis:** Greater intake of flavonoids, is associated with cIMT and carotid plaque composition, as measured by carotid ultrasound and MRI, respectively.

Project 2: Is flavonoid intake associated with neuroimaging markers of brain aging?

Mechanisms underlying the relationship between diet, stroke and cognitive decline still need to be explored. The ability to assess neurovascular and neurodegenerative disease is largely limited to clinical findings, such as clinical diagnosis of stroke/TIA, self-reported stroke symptoms in the absence of a stroke or TIA diagnosis, as well as cognitive dysfunction measured by neuropsychiatric assessment. As neuroimaging develops, especially magnetic resonance imaging (MRI), it is possible to quantify visible structural changes in the brain. Neuroimaging markers of cerebrovascular disease, such as brain volume, subclinical infarcts and increased burden of white matter hyperintensities may be more sensitive than clinical outcomes and are useful predictors of stroke.[314-316] Brain volume changes have been used as surrogates in clinical studies of Alzheimer's disease and can predict rapidity of cognitive decline in certain populations.[317]

Subclinical infarcts are typically punctate or circumscribed hypointense lesions on T1-weighted MRI scans. They are often similar to lacunar infarcts and likely develop due to small-vessel cerebrovascular disease. Subclinical infarcts that are larger than lacunes may reflect disease of larger vessels. Silent infarct burden increases with age, hypertension and other cardiovascular risk factors and these lesions are also tightly related with clinical strokes and cognitive decline.[207,318,319] Very few dietary studies have considered silent infarcts as outcomes. No published studies have examined fruit and vegetable consumption or reported flavonoid intake and their relationship with subclinical infarcts.

One study examined the association between adherence to a Mediterranean-style diet and MRI cerebral infarcts and found 40% fewer infarcts on the MRIs of participants with high adherence to a Mediterranean diet. The strength of the association was similar to that of hypertension and was not attenuated when the sample was restricted to those without baseline dementia or clinical strokes. The Mediterranean diet is likely beneficial, in part, through mechanisms that do not necessarily include fruit and vegetable intake, such as dietary fatty acid composition. However, given the beneficial associations between fruit and vegetable intake and cardiovascular health, the relationship between plant-based diets, flavonoid intake and subclinical disease burden should be investigated.

White matter hyperintensities (WMH), also known as leukoaraiosis, are typically bilateral patchy or diffuse areas of hyperintensity in the white matter of the brain seen on T2-weighted MRI images and on FLAIR sequences. While their pathogenesis is not clear, the severity of WMH increases with age and WMH are thought to reflect small vessel disease in the brain, resulting from ischemic damage due to chronic hypoperfusion. WMH can be seen in people who age normally, however, they are also associated with vascular risk factors such as smoking, diabetes, hypertension and dyslipidemia. They are associated with small vessel damage in other organs such as the eye and kidneys and heavy WMH burden is associated with an increased risk of stroke and dementia.[316,320-322] While a Mediterranean-style diet has been associated with lower WMH burden in a multiethnic cohort, in the Cardiovascular Health Study,[323] no existing published literature has examined the association between fruit and vegetable or flavonoid intake and WMH burden. It is possible that the pleiotropic effects of flavonoids, including preservation of

endothelial function, protection against neuroinflammation and reduction of cardiovascular risk factors, may also reduce the burden of WMH.

Structural changes in brain volume may be helpful to predict cognitive decline.[317] Neuronal loss, measured as changes in cortical thickness, which spreads to particular regions of the brain, such as the posterior cingulate and parieto-temporal cortices can be detected on MRI prior to dementia onset and also correlates with disease progression.[324-326] Though total brain and hippocampal volume have often been used as surrogate endpoints, ventricular volume trajectory may be the strongest predictor of Alzheimer's disease and vascular disease neuropathology. Increased volume of the cerebral ventricles, which produce and store cerebrospinal fluid, may combine loss of gray matter (neuronal loss) and white matter (axonal) with age-related changes in cerebrospinal fluid production and flow.[327] Again, high adherence to a Mediterranean-style diet is associated with decreases in cerebral atrophy,[328,329] but there are no other published studies that examine the association between fruit and vegetable or flavonoid intake and measures of cerebral atrophy. **Hypothesis:** Greater dietary intake of flavonoids is associated with fewer neuroimaging markers of brain aging, including brain volume, subclinical infarct burden and presence of white matter hyperintensities.

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