A Sex-Specific Approach to Stroke Epidemiology and Prevention

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CURRICULUM VITAE

Dr. Tracy Madsen is an emergency medicine physician and researcher dedicated to advancing the knowledge of sex and gender differences in stroke and translating this knowledge into improved stroke outcomes.

Her research interests began with a passion for women's health during her formative undergraduate and medical school years and evolved during her emergency medicine residency when she began investigating how sex and gender affect acute aspects of diseases such as sepsis and acute coronary syndrome. Following residency, she obtained formal research training through the completion of a research fellowship focused on sex and gender differences in disease. As part of her research fellowship, she earned a Master of Science in Clinical and Translational Research at the Brown University School of Public Health and used the skills to begin exploring sex and gender differences in cerebrovascular disease including knowledge of stroke symptoms, emergency department care, and eligibility for thrombolytics.

Her career objectives are to become a clinical epidemiologist and independent physician-scientist with a specific focus on assessing sex-specific stroke incidence, differences in stroke risk factors, and mechanisms of cerebrovascular disease by sex including the role of novel biomarkers and to translate sex differences in stroke risk into clinical tools that have the potential to improve stroke prevention strategies, reduce stroke burden, and improve health across diverse populations.

Her scientific contributions to date are numerous. Notably, her work has elucidated critical sex differences in nationally representative estimates of stroke incidence over time and has been published in high impact journals including *Neurology* and *Stroke*. She initially reported that overall, stroke incidence over time is decreasing to a lesser extent in women

compared with men and subsequently demonstrated that sex differences were most prominent in the oldest age groups, with differing patterns in younger age groups. Her work reporting an inverse association between sex hormone binding globulin, a sex steroid transporter, and incident stroke has contributed to knowledge of endogenous sex steroids and stroke. Finally, she demonstrated that the associations between elevated blood pressure and stroke differ by sex, a key finding toward more personalized prevention strategies.

Dr. Madsen's academic achievements and honors are also numerous. She has published over 60 peer-reviewed manuscripts and has had her work highlighted at international conferences including the International Stroke Conference as well as in the news media, notably *The New York Times* and *ABC News*. She is a Fellow of the American Heart Association as well as the American College of Emergency Physicians and has been appointed to numerous national taskforces. These include the Acute Care Task Force of the NINDS Brain Attack Coalition, the Stroke Workgroup of the ACEP Emergency Quality Network, and the NIH StrokeNet Prevention Working Group. She has served as an NIH early career reviewer and has been subsequently invited to serve on other NIH study sections. She also serves as a reviewer for several high impact journals including *Stroke, Neurology*, *Annals of Epidemiology*, and *Lancet* and was recently invited to serve as an editorial board member for *Neurology*. In addition to an NHLBI career development award, she has received funding from two foundation grants to study sex differences in stroke, the Rhode Island Foundation Medical Research Grant and the Robert E. Leet and Clara Guthrie Patterson Trust Mentored Research Award.

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INTRODUCTION

Stroke is a leading cause of disability and death in the United States (U.S.) and globally.[1] In the U.S., 795,000 strokes occur every year; stroke is the 4th leading cause of death for women and the 5th leading cause of death for men,[2] and is among the leading causes of disability.[1] Globally, stroke is the third leading cause of disability, accounting for 5.7% of disability adjusted life years, defined as the number of years lost to illness, disability, or early death.[3] Finally, in the U.S. alone, the yearly direct costs of stroke exceed $23 billion.[4]

Despite the fact that stroke is a leading cause of death and disability for both sexes, women experience a disproportionate amount of the stroke-related disease burden. Due largely to the increasing incidence of stroke with age as well as a longer life expectancy for women, women have a higher lifetime risk of stroke and more deaths from stroke compared with men;[1] women also have worse outcomes following stroke and are more likely to have resulting disability and dependence.[5,6]

Sex differences in the epidemiology of stroke must be continuously re-evaluated to understand the changing impact of stroke on population health over time and to remain aware of opportunities to improve targeted primary and secondary prevention. For example, similar to findings from the Atherosclerotic Risk in Communities study,[7,8] my own work has shown that stroke incidence has decreased since the early 1990s in the U.S., though temporal trends vary by sex, age, and stroke subtype.[9] With regard to differences by sex and age category, my recent work with the Greater Cincinnati Northern Kentucky Stroke Study (GCNKSS) demonstrated that stroke incidence among those 65 to 84 years old decreased between 1993 and 2015 in both women and men, while stroke incidence among those ≥ 85 years old decreased in men only, and stroke incidence among those 20-44 years old increased in men but were stable in women.

Further, this work demonstrated that decreasing incidence rates of stroke are driven by decreases in acute ischemic stroke (IS) as opposed to hemorrhagic subtypes.[9]

To explain sex differences in the distribution of stroke at the population level, risk factors must be evaluated in a sex-specific manner. Not only could sex differences in stroke risk factors help to understand disparities in preventative efforts, but a more complete understanding of risk factors by sex could also advance our understanding of how the risk of stroke changes over the life course. For example, data have largely shown a higher stroke risk in men compared with women throughout early and midlife; stroke rates in women often catch up to or surpass stroke rates in men beginning with the eighth decade of life.[9,10] Other data conflict to some degree and indicate that stroke risk among women of reproductive age may exceed those of men.[11] Sex-specific risk factors that contribute to stroke in younger and even middle age women include pre-eclampsia and migraine with aura,[12,13] though more data on mechanisms behind these risk factors are needed. Sex differences in the relations between diseases such as atrial fibrillation and diabetes mellitus and stroke have also been well-described and are characterized by a stronger association with stroke in women compared with men.[14–16] Such literature emphasizes the need to continue to evaluate stroke risk factors in a sex-specific manner and to consider sex-specific guidelines for particular risk factors as more data emerge.

As our knowledge of sex differences in stroke epidemiology increases, we must simultaneously improve our understanding of the biologic basis for sex differences in stroke phenotypes. For example, while it is clear that stroke risk rises dramatically with age in women, the specific relationship of stroke risk to menopause is less clear. It has long been speculated that endogenous sex steroids such as estradiol and progesterone are protective against stroke and cardiovascular disease (CVD), though exogenous administration of such hormones resulted in an

increase in risk of stroke and coronary heart disease.[17] More studies are needed to understand the role of female sex hormones in the changing risk of stroke over the life course and subsequently how preventative strategies in women might be improved based on the mechanistic role of sex hormones.

The overall objective of my thesis is to advance our understanding of the sex-specific contribution of various risk factors, to examine the hormone-related biologic basis for sex differences in stroke, and to apply the new insights gained to the creation of a sex-specific stroke risk score. This thesis contains four chapters corresponding to the following three specific aims, respectively.

Aims:

Aim 1 (Chapters 1-2): To investigate whether there are sex differences in the association between two major modifiable stroke risk factors, hypertension and diabetes mellitus, and incident ischemic stroke in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study.[18]

Aim 2 (Chapter 3): To determine whether there is an association between low sex hormone binding globulin (SHBG) and the risk of incident IS using previously collected data and measurements from women in the Women's Health Initiative (WHI) study.[19]

Aim 3 (Chapter 4): Using a cohort of participants from the WHI, to create a sex-specific risk score for IS in women using conventional stroke risk factors, risk factors unique to women (i.e., migraine, reproductive risk factors), and SHBG as a novel biomarker.

CHAPTER 1: The Sex Specific Relations between Modifiable Risk Factors and Stroke: Hypertension

Abstract:
Little is known about whether the relationship between hypertension and ischemic stroke (IS) differs by sex. We examined sex differences in the association between hypertension severity and treatment and IS risk. We used data from REGARDS, a longitudinal cohort study in the continental United States, with oversampling of Black individuals and those living in the stroke belt. We included 26,461 participants recruited from 2003-2007 without prevalent stroke at baseline. The main outcome was incident IS ascertained by telephone surveillance (with physician adjudication for suspected events). Proportional hazards regression was used to assess the sex-specific association between systolic blood pressure and stroke and between classes of antihypertensive medications and stroke after adjustment for age, race, sex, and age-by-race and sex-by-treatment interaction terms. *A priori,* $p< 0.10$ was considered significant for interactions. Among participants (55.4% women, 40.2% Black), there were 1084 confirmed ischemic stroke events. In the adjusted model, the risk of stroke per each level of hypertension (referent/ systolic blood pressure <120 mm Hg/ 120-129 mm Hg/ 130-139 mm Hg/ >140 mm Hg) was higher in women (HR 1.25, 95% CI 1.16-1.34) than men (HR 1.14, 95%CI 1.05-1.23) (sex-systolic blood pressure interaction term, $p = 0.09$). Compared to no medications, with each additional class of medications, stroke risk increased by 23% (HR 1.23, 95%CI 1.14-1.33) for women and 21% (HR 1.21, 95%CI 1.12-1.31) for men ($p=0.79$). Further work on the biological mechanisms for sex differences in stroke risk associated with hypertension severity, particularly in evaluating the clinical effectiveness of sex-specific clinical guidelines is warranted.

Introduction

There are substantial sex differences in age-adjusted stroke incidence,[1,12] stroke prevalence,[1,12] and in the prevalence and risk associated with cardiometabolic factors including hypertension, atrial fibrillation, diabetes, and smoking.[16] An improved understanding of these differences is needed to ensure that stroke prevention strategies are effective for both women and men. Despite higher age-adjusted stroke rates in men in many age categories, women have more strokes and more deaths from stroke than men over the lifetime,[1,20,21] thought to be largely due to differences in life expectancy.[22] Conflicting data exist in the literature concerning the incidence of stroke in recent years between women and men. While some work suggests that stroke incidence has been decreasing faster in men than women from 1993 to 2010,[23] others have shown similar rates of decline in stroke incidence for men and women.[8,24] One potential contributor to sex differences in stroke incidence over time and over the lifespan is a sex difference in the way co-morbidities such as hypertension affect stroke risk.[16]

Hypertension, the most common modifiable stroke risk factor, is known to differ in prevalence, rates of control, and degree of associated stroke risk between women and men. The prevalence of hypertension differs by sex; prevalence is lower in women than men under 60 years of age but higher in women than men after that time point.[25] Control of blood pressure (BP) also differs by sex and across the lifespan, as women in older age groups are less likely to have their hypertension controlled compared with men.[25,26] Finally, data on the degree of stroke risk associated with hypertension conflict to some extent, with some data showing similar estimates of the association between increasing hypertension and stroke risk[27–29] and other showing potentially differing estimates between hypertension and stroke by sex.[30] Race and ethnicity play an important role in hypertension prevalence and control as well. The overall age-adjusted prevalence of hypertension is higher in Black women than all other race/gender

subgroups, and control of hypertension is lower among non-white and Hispanic women compared to white, Non-Hispanic women.[25] Previous work in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study found a sex difference in the association of systolic blood pressure (SBP) and use of antihypertensive medications with risk of ischemic stroke in whites but not Blacks.[10] Finally, though it is well known that hypertension is also a risk factor for hemorrhagic stroke, the focus of the current paper is on IS given the significant differences in pathophysiology between ischemic and hemorrhagic strokes as well as low numbers of hemorrhagic strokes in the cohort.

The primary objective of our study was to determine whether there are sex differences in the association between hypertension severity or the treatment of hypertension and risk of IS.

Methods

Study Population

The study population included participants in the REGARDS study, a prospective, national, longitudinal cohort study of 30,239 non-Hispanic Black and white adults recruited between January 2003 and October 2007. Adults aged 45 years and older were enrolled from across the continental U.S., with oversampling of Black individuals and those living in the stroke belt (North Carolina, South Carolina, Georgia, Tennessee, Alabama, Mississippi, Arkansas and Louisiana). Further details of REGARDS methodology can be found elsewhere.[31] For the current analysis, we included data from 26,461 participants after excluding 1841 due to prevalent stroke at baseline, 1467 due to missing data on medication inventory/ inability to determine number of anti-hypertensive medications, 414 with no follow-up, and 56 because of data anomalies (Figure 1.1).

Collection of Exposure and Outcome Data/ Definitions of Exposure and Outcome

Baseline data on patient demographics and use of anti-hypertensive medications were collected through a telephone interview followed by an in-home visit.[31] A physical exam including BP measurements was also completed during the in-home visit. Mean systolic blood pressure (SBP) was calculated from 2 consecutive measurements on the same arm, taken by a trained technician after the patient had been sitting quietly for at least 5 minutes. The protocol for measuring SBP was standardized, and measurements were taken using an aneroid sphygmomanometer that was regularly tested. Most (91%) of the blood pressure measurements were generally taken between 7 AM and 12 noon. Blood pressure measurements were taken in the left arm unless not possible, and a large cuff was used for participants with arm circumference greater than 13 inches. For actual measurements, the cuff was inflated 20 mmHg above the pulse obliteration level and then deflated at a rate of approximately 2mm Hg/second. Throughout the study, blood pressure quality control was monitored centrally for potential digit preference, and study staff were retrained as needed. Surveillance for suspected IS events, the primary outcome in this study, was conducted via computer assisted telephone interviews that occurred every 6 months. Medical records for suspected stroke events were retrieved, and possible events were adjudicated by trained study physicians. Based on review of the medical record including imaging results, physicians first adjudicated suspected events as cases or not cases and then subtyped the cases into ischemic or hemorrhagic. Based on the World Health Organization (WHO), the definition of stroke is "rapidly developing clinical signs of focal, at times global, disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin." Strokes that didn't meet the WHO definition but that met REGARDS clinical criteria were also included (symptoms suggestive of stroke with unconfirmed duration or inconclusive imaging; or imaging definitive for stroke

without typical symptoms). Those that were not primarily hemorrhagic based on physician review of medical record and imaging results were categorized as ischemic. Only IS were included in this analysis. Additional details of the adjudication process can be found in prior publications.[32]

Our primary exposure variables were sex, strata of hypertension severity, and number of classes of anti-hypertensive medications that participants reported at the index home visit. Hypertension severity was assessed in two ways, as a continuous variable and as an ordinal variable based on the 2017 American College of Cardiology (ACC)/ American Heart Association (AHA) Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults as follows: normotensive (<120 mm Hg) (reference group)), elevated BP (120-129 mm Hg), Stage 1 hypertension (130-139 mm Hg), and Stage 2 hypertension (>140 mm Hg).[33] Medications reported by participants at the baseline visit were classified as anti-hypertensives by trained research staff and categorized into the following classes: angiotensin-converting enzyme inhibitors, aldosterone antagonists, α-blockers, angiotensin II receptor blockers, β-blockers, calcium channel blockers, central agonists, diuretics, or vasodilators. Participants who reported not taking any antihypertensive medications were the reference group, and the other categories were 1, 2, or 3 or more classes of anti-hypertensive medications. The study was approved by the institutional review board of each institution.

Data Analysis

Descriptive statistics (frequencies, proportions) were used to compare basic demographics (age, race) by sex. Descriptive statistics (frequencies, proportions, means with standard deviations as appropriate) were also used to determine the prevalence of each stratum of hypertension within sex and race subgroups.

Proportional hazards regression was used to determine both the association of the level of hypertension severity (normal, elevated, stage 1, or stage 2) and the number of classes of anti-hypertensive medications with incident IS. Two product terms were included in the model: Sex-by-SBP category and number of medications-by-SBP category. Models were also adjusted for age, race, and an age-by-race interaction term, which has been shown to be significant in prior REGARDS studies.[32] Sex-specific estimates of stroke risk were calculated for each stratum of hypertension severity and number of classes of anti-hypertensive medications. Within each sex, trends in stroke risk across increasing BP severity strata and across increasing number of medication classes were tested for significance. In addition, effect estimates for stroke risk between sexes were compared by testing for heterogeneity. These analyses were repeated in a race-stratified manner as well. As a sensitivity analysis, models were further adjusted for the remaining Framingham stroke risk factors given that they may be confounders of the association between SBP and stroke: history of diabetes, current atrial fibrillation, current smoking, and prevalent cardiovascular disease. Though it is a Framingham risk factor, left ventricular hypertrophy was not included in the model as it is likely a consequence of SBP and therefore not a confounder.

These analyses were repeated with SBP as a continuous variable to determine the sex-specific increase in stroke risk with 10 mmHg incremental increases in SBP, adjusting for age, race, and an age-by-race interaction term.

With regard to censoring, 1084 had a stroke endpoint, and 25,377 were censored. Of these, 5505 died of another cause prior to having a stroke event (and were censored at the time of their death), 5831 had withdrawn from the study (and were censored at the last contact where

they were known to be stroke-free), 14040 were considered to be in active follow-up (also censored at their last stroke-free contact), and 1 participant had a follow-up status in review.

For all models, adjusted hazard ratios with 95% CI were reported separately for women and men. Chosen *a priori*, an alpha of 0.05 was used for main effects. As per prior REGARDS analyses, an alpha of 0.10 was chosen *a priori* for tests of interaction in order to reduce the likelihood of missing a significant interaction term and reporting averaged associations for men and women when the true associations differ by sex. In addition, compared with main effects, detecting group differences using interaction terms requires much larger sample sizes; choosing alpha values that are slightly higher may partially compensate for this issue.

Results

Of 26,461 participants included in the analysis (Figure 1.1), 55.4% (n=14668) were women, and 40.2% (n=10644) were Black. Mean age was similar between women and men (64.2 (SD 9.5) versus 65.5 (SD 9.3) years). Over a mean follow-up period of 8.7 +/- 3.6 years, there were 1084 incident IS events. Table 1.1 shows SBP (as mean (SD) and by categories) along with the number of IS events in each demographic group. Compared with men, there was a higher proportion of women participants in the normotensive group and slightly lower proportions in the Stage 1 and Stage 2 hypertensive groups.

Table 1.2 displays sex-specific estimates of the risk of IS associated with increasing SBP by category, stratified by number of classes of antihypertensive medications; information is displayed graphically in Figure 1.2. For both sexes, in all strata of number of anti-hypertensive medications, the IS risk increased with increasing SBP. Effect sizes, however, were often greater for women than men, most clearly demonstrated by higher risk estimates in 3 of 4 strata of number of classes of antihypertensives. For example, for those participants on 3 classes of

medications, the average increase in IS risk per BP level was 1.26 (95% CI 1.07-1.47) for women and 1.07 (95% CI 0.91-1.26) for men (p=0.18). Average risk increases were not significantly different by sex in individual strata. The overall pooled effect estimates for women vs. men were1.25 (95% CI 1.16-1.34) vs. 1.14 (95%CI 1.05-1.23), respectively, with a test for interaction reaching a priori statistically significant alpha of 0.10 (p=0.09). When SBP was treated as a continuous variable, women had higher risk of stroke per 10 mm Hg increase in SBP as indicated by larger effect estimates (1.15, 95%CI 1.10-1.20, vs. 1.08, 95%CI 1.03-1.14, P=0.09). The data in Table 1.2 also show increasing stroke risk with increasing numbers of anti-hypertensive medications in all strata of SBP. Pooled effect estimates of the average increase in stroke risk per increase in number of medications were similar by sex (1.23, 95%CI 1.14-1.33 for women, 1.21 95%CI 1.12-1.31 for men, p=0.79).

Appendix Table A1 displays the prevalence of additional Framingham stroke risk factors by sex and systolic blood pressure category, and Appendix Table A2 displays sex-specific estimates of the risk of IS associated with increasing SBP by category, stratified by number of classes of antihypertensive medications and adjusted by the four additional Framingham risk factors: diabetes, atrial fibrillation, current smoking, and prevalent cardiovascular disease. In this model, the overall pooled hazard of stroke with increasing SBP was 1.24 (95%CI 1.15-1.35) for women vs. 1.13 (95%CI 1.04-1.23) for men and was significant at p=0.08.

Appendix tables A3 and A4 show the sex-specific effects of increasing hypertension severity by strata of hypertension across strata of number of anti-hypertensives in Black and white participants separately. Among Black participants, the pooled effect estimate of increased stroke risk per increasing level of hypertension severity was 1.26 for women (95%CI 1.13-1.41) and 1.19 for male participants (95%CI 1.03-1.36, p=0.51) (Table A3). Among white participants,

the pooled effect estimate of increased stroke risk per increasing level of hypertension severity was 1.24 for women (95%CI 1.11-1.38) and 1.12 for male participants (95%CI 1.02-1.23, p=0.17) (Table A4).

Discussion

In this large, national, prospective cohort study, the association between increasing hypertension severity and incident IS was almost twice as large in women compared with men. This sex difference remained after adjustment for other conventional stroke risk factors. While further research is needed to confirm our findings in other study populations and to investigate potential pathophysiologic differences in the link between hypertension and cerebrovascular disease, our findings suggest that a sex-specific approach to BP control and risk factor modification should be considered.

Few prior studies have been designed to detect sex differences in the association between hypertension severity and stroke risk either in the study design or data analysis phases, and those that have reported sex-specific associations between stroke risk and BP have shown conflicting results.[10,27,30,34] Our study adds to the current literature by evaluating this question in a large prospective cohort with both White participants of European ancestry, and Black participants of African ancestry, by taking into account the number of medications used to treat the hypertension, and by using the 2017 ACC/AHA High BP Guideline with 120 mmHg as the threshold for a diagnosis of hypertension.[33] Another recent study from REGARDS also demonstrated a larger association between factors such as hypertension, diabetes, heart disease and stroke events in women compared with men, though these differences were only present among whites.[10] Contributors to this race difference are unclear but could be related to higher underlying prevalence of both comorbidities and strokes among Black individuals. A previous

large study of older adults demonstrated a stronger association between hypertension and stroke in women compared with men, but the severity of hypertension was not assessed.[30] A secondary analysis of SPRINT data that assessed sex-specific effects of intensive BP lowering on the composite outcome of CVD events also found sex differences. The effect estimate included 1.0 for women but not for men, suggesting a statistically significant benefit for men but only a trend towards a significant benefit for women. The p-value for the interaction was non-significant.[34] Women, however, were underrepresented in SPRINT, comprising only 35% of participants. A large meta-analysis of studies that reported sex-specific associations between SBP and stroke risk found similar risks for women and men, but did not account for possible treatment differences.[27] These studies, in conjunction with our current findings of a stronger association between hypertension severity and IS in women compared with men, suggest the need for future well designed studies powered to investigate possible sex specific effects of hypertension on stroke risk.

Possible explanations for our finding that hypertension severity carries a greater stroke risk for women than men are numerous and require further consideration. First, biologic and/or hormonal mechanisms that link hypertension to vascular dysfunction and disease may well differ between the sexes. For example, there is evidence that beta blockers are less effective in reducing sympathetic nerve activity and peripheral vascular resistance in postmenopausal women compared with men.[35] Second, sex specific synergistic effects between hypertension and other risk factors such as diabetes may also contribute to vascular dysfunction and thus increase stroke risk.[30] In a large study of Taiwanese adults over age 65, the combined effect of having hypertension and diabetes on stroke risk was greater for women than men.[30] Third, sex difference in hypertension treatment and adherence is also a possibility.

Our finding of a stronger association between stroke risk and increasing hypertension severity in women vs. men suggests that, pending future confirmation of such sex differences, sex-specific hypertension guidelines for stroke prevention may be warranted. The SPRINT trial demonstrated a statistically non-significant benefit of controlling SBP to < 120 mm Hg compared with the prior target of < 140 mm Hg in women. Due to issues with statistical power, however, based on these data, it is unknown whether the threshold for treating hypertension should be equivalent in men and women. Future trials should be designed with sufficient power to adequately assess treatment effects in each sex. Additionally, we need more data from both pre-clinical and clinical research to assess how risk factors like diabetes and hypertension act synergistically to increase cardiovascular disease and stroke risk in women vs. men. Further studies of the role that endogenous sex hormones may play in the association between hypertension and stroke in women should also be considered.

It is unclear why there might be a sex difference in stroke risk associated with increasing hypertension severity among white participants but not Black participants. There are differences in the distribution of hypertension severity by race that must be considered. In our dataset, overall there were fewer Black men than all other subgroups. Compared to white women and men, Black women and men had more severe hypertension and were less likely to be in the normotensive category. Treatment disparities could also be a factor; one could speculate that if BP over time is treated more aggressively in white men compared with other race/sex subgroups, a sex difference would be seen between white men and women but not between Black men and women. Finally, there may be confounding variables (i.e. socioeconomic status and health insurance) that affect the association between BP and stroke risk in Black and white participants for which we did not account in our study.

We found that, in general, at a fixed level of BP, the risk of IS increased with the number of classes of anti-hypertensive medications reported by participants, even among normotensive individuals. Our data do not suggest that stroke risk increases with use of larger numbers of antihypertensive medications, but rather that stroke risk is lower if a person requires a fewer number of medications to maintain a specific BP level. This is consistent with our prior data,[36] but in the present study, our findings additionally demonstrate no sex differences in the impact of the number of antihypertensive medications on stroke risk. Our findings of increased IS risk with an increasing number of medications, even among those in the normotensive category, suggest that controlling BP with medication does not fully mitigate the biologic and/or physiologic mechanisms that contribute to stroke risk among those diagnosed with hypertension. Such factors are important targets for future interventions. Our findings further suggest that most efficient approach for BP control is the primordial prevention of hypertension. At least 5 approaches with class 1-A evidence have been shown to prevent or delay the onset of hypertension.[37,38]

Our study has several limitations. First, we were unable to investigate the effect of changing hypertension severity over time, as our BP measurements were taken at the time of study enrollment only. Our study can be used to assess the sex-specific association between SBP at a given time point and subsequent stroke risk, which is of importance when considering strategies to reduce stroke risk across populations. Another limitation of our study is that, though we adjusted for the effect of age on the relationship between hypertension severity and stroke risk, we did not report risk estimates in specific age groups because of the small numbers of participants in the various age, sex, and race-specific strata. We could also not adjust for age at menopause. In addition, though we investigated the effect of increasing numbers of anti-

hypertensive medications on stroke risk, this study was not designed to address adherence with medications or adjustment of medications over time, which could vary by sex and race. Studying provider and patient behaviors around the treatment of hypertension over time is an important future research direction. Despite these limitations, the design of REGARDS as a longitudinal cohort study of almost 30,000 individuals , the oversampling of Black individuals, and the availability of adjudicated stroke events make REGARDS an ideal study to evaluate the association between hypertension severity and stroke. Finally, the focus of our manuscript was the association between SBP and ischemic stroke, elevation of which is known to be associated with increased risk of cardiovascular disease and stroke. There may also be sex differences in the way in which diastolic blood pressure and pulse pressure affect stroke risk,[27-28] a topic for future research. Hemorrhagic strokes were not included in our study given the clear pathophysiologic differences between IS and hemorrhagic stroke though should also be evaluated in a separate study with sufficient number of strokes to evaluate this topic.

Conclusions

In summary, the relationship between severity of hypertension and risk of IS was greater in women than men. Treatment with additional classes of antihypertensive medications was associated with increased IS risk in both women and men. Further work on the biological mechanisms of sex differences in severity of HTN, and its CVD complications, including stroke, and the potential need for sex-specific clinical guidelines for hypertension prevention and treatment is warranted.

Tables and Figures

Table 1.1: Systolic Blood Pressure and Stroke Outcomes, by Sex and Race Categories

Characteristic	Women (n=14,668)			Men (n=11,793)		
	All (n=14668) n (%)	Black (n=6674) n (%)	White (n=7994) n (%)	All (n=11793) n (%)	Black (n=3970) n (%)	White (n=7823) n (%)
Systolic blood pressure (mm Hg) (mean, SD)	126.0 (16.8)	129.5 (7.2)	123.0 (15.8)	128.5 (15.9)	131.5 (16.7)	126.9 (15.3)
Systolic Blood Pressure (mm Hg)						
Normotensive (<120)	5121 (34.9)	1793 (26.9)	3328 (41.6)	3328 (28.2)	860 (21.7)	2468 (31.5)
Elevated (120-129)	3985 (27.2)	1774 (26.6)	2211 (27.6)	3277 (27.8)	1025 (25.8)	2252 (28.8)
Stage 1 (130-139)	2834 (19.3)	1492 (22.3)	1342 (16.8)	2653 (22.5)	985 (24.8)	1668 (21.3)
Stage 2 (>140)	2728 (18.6)	1615 (24.2)	1113 (13.9)	2535 (21.5)	1100 (27.7)	1435 (18.3)
Ischemic stroke events	542	269	273	542	179	363

Table 1.2: Sex-specific Hazard Ratios Linking Systolic Blood Pressure and Number of Classes of Blood Pressure Medications to Risk of Ischemic Stroke

Systolic blood pressure		No Medications	1 class of medications	2 classes of medications	3 classes of medications	Change per class, and p-value for effect modification by sex	Pooled effect of medications,* and p-value for effect modification by sex
Normal	W	1.00 (ref)	1.29 (0.78 – 2.13)	2.18 (1.38 – 3.44)	2.80 (1.71 – 4.60)	1.43 (1.23 – 1.67)	
	M	1.00 (ref)	1.17 (0.68 – 1.98)	1.54 (0.92 – 2.56)	2.62 (1.59 – 4.34)	1.36 (1.15 – 1.60) P = 0.64	W: 1.23 (1.14 – 1.33) M: 1.21 (1.12 – 1.31) P = 0.79
Elevated	W	1.11 (0.69 – 1.79)	1.77 (1.12 – 2.80)	1.91 (1.22 – 3.00)	1.45 (0.81 – 2.60)	1.13 (0.95 – 1.34)	
	M	1.18 (0.75 – 1.85)	2.20 (1.43 – 3.40)	1.85 (1.16 – 2.95)	1.73 (1.01 – 2.96)	1.14 (0.98 – 1.32) P = 0.96	
Stage 1	W	1.82 (1.12 – 2.97)	2.56 (1.64 – 3.99)	2.07 (1.31 – 3.27)	3.55 (2.25 – 5.60)	1.19 (1.01 – 1.40)	
	M	1.44 (0.90 – 2.31)	1.68 (1.05 – 2.70)	2.31 (1.48 – 3.60)	2.88 (1.82 – 4.57)	1.27 (1.10 – 1.48) P = 0.54	
Stage 2	W	2.56 (1.58 – 4.15)	2.74 (1.79 – 4.19)	2.86 (1.86 – 4.38)	4.20 (2.76 – 6.39)	1.18 (1.02 – 1.37)	
	M	2.07 (1.31 – 3.28)	1.66 (1.03 – 2.68)	2.30 (1.47 – 3.60)	2.75 (1.75 – 4.32)	1.13 (0.97 – 1.31) P = 0.68	
Change per class	W	1.38 (1.18 – 1.62)	1.29 (1.11 – 1.49)	1.10 (0.95 – 1.28)	1.26 (1.07 – 1.47)		
P-value for effect modification by sex	M	1.27 (1.09 – 1.47) P = 0.44	1.06 (0.90 – 1.23) P = 0.069	1.14 (0.99 – 1.33) P = 0.72	1.07 (0.91 – 1.26) P = 0.18		
Pooled effect of BP†	W	1.25 (1.16 – 1.34)					
	M	1.14 (1.05 – 1.23)					
		P = 0.091					

W: Women; M: men; BP: blood pressure; Effect estimates are hazard ratios adjusted by age, race, age-by-race interaction term.

*Across strata of BP, 3-degree freedom test for differences within sex, p=0.15 (women), p=0.28 (men)

† Across strata of medication classes, 3-degree freedom test for differences within sex p = 0.22 (women), P=0.31 (men)

Figure 1.1 Final Analytical Cohort of Participants from the Original REGARDS Study Sample

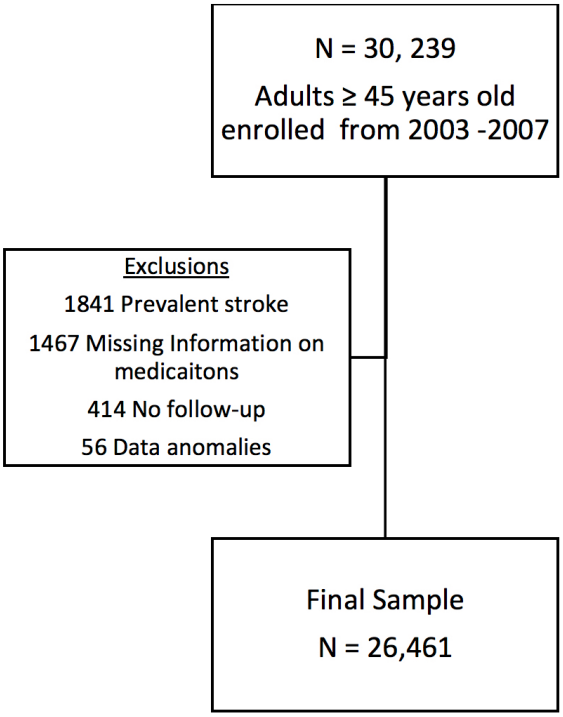
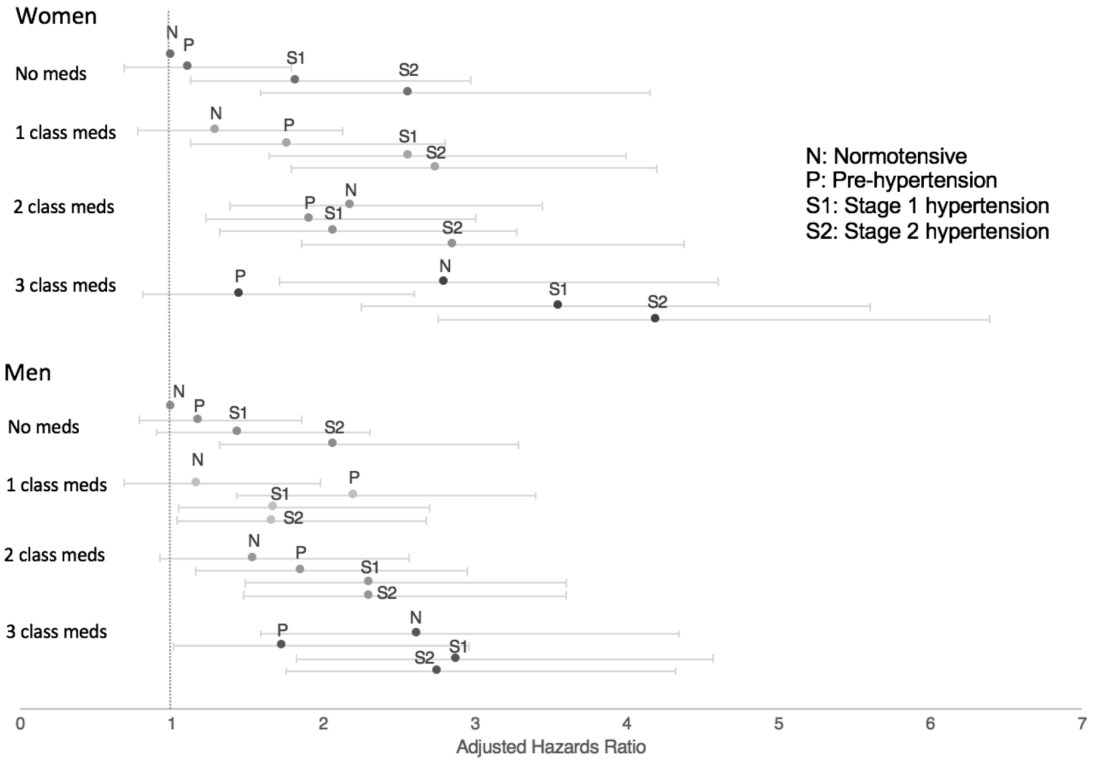


Figure 1.2 Sex-specific Hazard Ratios Demonstrating Association between Increasing Systolic Blood Pressure, Number of Classes of Blood Pressure Medications, and Risk of Ischemic Stroke



Within each grouping of the number of medications, the four data points correspond to normotensive (<120 mmHg), pre-hypertension (120-129 mmHg), stage 1 (130-139 mm Hg) and stage 2 (>140 mm Hg) blood pressure strata. (Hazard ratios adjusted by age, race, and age-by-race interaction term)

CHAPTER 2: The Sex Specific Relations between Modifiable Risk Factors and Stroke: Diabetes and Impaired Glucose Metabolism

Abstract

In this chapter, we aim to investigate whether glycemic control and impaired glucose tolerance accounts for sex and race differences in the risk of ischemic stroke (IS) associated with diabetes mellitus (DM). This prospective longitudinal cohort study included adults age ≥45 years at baseline enrolled in the Reasons for Geographic And Racial Differences in Stroke Study (REGARDS) and followed for a median of 11.4 years. The exposure was baseline fasting blood glucose (FBG) (mg/dL); suspected IS events were ascertained by phone every 6 months and physician-adjudicated. Cox proportional hazards were used to assess the adjusted sex/race-specific associations between FBG and incident IS. Of 20,338 participants, mean age was 64.5(SD 9.3) years, 38.7% were Black, 55.4% were women, and 16.2% were using DM medications. There were 954 IS events. Compared to FBG <100, FBG ≥150 was associated with 59% higher hazards of IS (95%CI 1.21-2.08) and 61% higher hazards of IS when restricting to those on DM medications (95%CI 1.12-2.31). Overall, the association between FBG and IS varied by race/sex (HR for FBG ≥ 150 vs. FBG <100: white women 2.05 (95% CI 1.23-3.42), Black women 1.71 (95%CI 1.10-2.66), Black men 1.24 (95%CI 0.75-2.06), white men 1.46 (95%CI 0.93-2.28), $p_{interaction}$=0.004). Similar findings by race/sex were obtained among those using DM medications. The magnitude of the association between increasing FBG and IS was higher among white women and Black women compared with white men and Black men, suggesting sex differences in the role of impaired glucose metabolism in stroke risk.

Introduction

There are known sex differences in the association between diabetes mellitus (DM) and risk of stroke with one meta-analysis showing that the sex-specific stroke risk associated with DM is approximately 30% higher for women than men.[15] Less is known about the association between DM and stroke by race and ethnicity, likely due to a lack of data or lack of diversity in study samples. More recently, sex- and race-specific analyses from the REGARDS study demonstrated that the stroke risk associated with DM varies by sex in white but not Black participants.[10,39] Finally, other literature has demonstrated that DM is associated with vascular-related mortality (a combination of ischemic heart disease and stroke) to a greater extent for women than men.[40]

Mechanisms behind these sex differences are not clear, though there is speculation that factors such as disparities in the treatment and/or control of DM or of co-existing risk factors including hypertension or hyperlipidemia may contribute.[41,42] When considering contributors to sex differences in particular, other data point to pathophysiologic differences between women and men, specifically that women with a clinical diagnosis of DM have accumulated a greater degree of endothelial dysfunction compared with men with DM.[43] Disparities in the treatment and control of cardiovascular risk factors by race could also be driving potential differences between demographic groups and must be addressed given known rates of elevated stroke risk and mortality among Black individuals.[41] Previously, studies evaluating sex and race-based differences in stroke risk associated with DM have not consistently accounted for use of diabetes medications, anti-hypertensives, or control of DM as measured by fasting blood glucose or hemoglobin A1c, another gap in the literature.[15,40]

Elevated fasting blood glucose (FBG), a marker of the severity of abnormal glucose metabolism as well as diabetes control, has been shown to be associated with an increased risk of cardiovascular disease (including stroke), though the relationship may be non-linear, and data conflict as to whether this relationship varies by sex.[44–46]

Our objectives were to determine: 1) the prevalence and severity of impaired glucose metabolism by sex/race subgroups; and 2) whether there are differences in the risk of incident IS across increasing levels of FBG between race/sex subgroups, accounting for the use of diabetes medications as well as other stroke risk factors.

Methods

Study Population/ Participants

The REGARDS study is a national longitudinal cohort study into which 30,239 adults ≥ 45 years of age were enrolled between 2003 and 2007; individuals who identified as Black and/or living in one of the eight stroke belt states were oversampled. Detailed study methodology has been previously published.[31] A home visit was conducted at the time of enrollment to collect data on demographics, medical history, and medications, including those for diabetes. Vital signs, electrocardiogram and a blood draw were also performed during the initial home visit. For this analysis, participants were excluded if they had prevalent stroke at baseline, did not have a FBG performed, or had missing data on any of the covariates used in the primary analysis (Figure 2.1). The REGARDS study was approved by institutional review boards at all participating sites. All participants provided written informed consent.

Exposure

The primary exposure variable was FBG measured during the initial home visit. Participants were asked to fast overnight for 10-12 hours, and blood glucose was measured using

colorimetric reflectance spectrophotometry on the Ortho Vitros 950 IRC Clinical Analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA) at the University of Vermont.

Outcome

The outcome was incident IS, defined as a sudden onset, neurologic deficit lasting ≥ 24 hours or imaging evidence of a stroke with accompanying appropriate symptoms, and with no evidence of intracranial hemorrhage as the primary stroke subtype. Potential cases of IS are initially identified via computer assisted telephone interviews that occur every 6 months. Self- or proxy-reported medical encounters suspected to be stroke events are then adjudicated by trained study physicians using medical records and results of brain imaging. For this analysis, events through September 2018 were included. Details of case ascertainment and adjudication have been published previously.[32]

Covariates

Data on the primary independent variables (biologic sex (male/ female) and race (Black/white)), were collected during interviews occurring at the time of study enrollment. Other demographic variables collected at baseline include age, education, and annual household income. Clinical variables included body mass index (BMI) as well as factors included in the Framingham Stroke Risk Score (baseline systolic blood pressure (SBP), use of anti-hypertensive medications, history of atrial fibrillation, left ventricular hypertrophy, history of heart disease, smoking status, and history of self-reported diabetes). Finally, whether women had ever used menopausal hormone therapy (MHT) was obtained by self-report.

Statistical Analyses

Baseline characteristics of the sample by categorical FBG level were analyzed using descriptive statistics (frequencies with proportions or means with standard deviations as

appropriate). Next, prevalence of hyperglycemia (defined here as FBG ≥ 150 or ≥200 mg/dL) was described by race/sex subgroup using frequencies and proportions.

Cox proportional hazards models were used to assess the association between FBG (<100 (ref), 100-125, 126-149, ≥150 mg/dL) and incident IS. Pooled hazard ratios (overall hazard ratios across all race/sex subgroups) followed by race and sex-specific hazard ratios for white women (WW), Black women (BW), white men (WM), and Black men (BM)) were obtained. Analyses were performed for the entire study sample (Model 1), followed by analyses stratified by use of DM medication (oral medications or insulin vs. no DM medication use) (Models 2 and 3). The four categories of FBG were chosen based on established clinical thresholds for impaired glucose metabolism: euglycemia (<100 mg/dL), pre-diabetes (100-125 mg/dL), clinical diabetes (≥126 mg/dL), and FBG ≥ 150 mg/dL to capture those with more severe disease. In each of the above models, in addition to including FBG and a race/sex by FBG interaction term, we adjusted for demographic variables (age and a interaction term for age by race/sex subgroup), variables corresponding to the Framingham Stroke Risk Score,[47] body mass index (BMI), education level, and annual household.

Adjusted hazards ratios with 95% confidence intervals representing the risk of IS specific to each race/sex subgroup across increasing FBG levels were reported with FBG < 100 as the reference group. We also reported the results of trend tests for IS risk across increasing FBG category and p-values for the race/sex by FBG interaction term. For main effects, an alpha value less than 0.05 was considered to be statistically significant; for interaction terms, an alpha value less than 0.10 was chosen *a priori*.

Additional Planned Analyses

We performed Cox proportional hazards regression with incident IS as the outcome, but with the inclusion of FBG as a restricted cubic spine term to assess the potential non-linearity of the relationship of FBG and incident IS. The RCS function had 5 knots with the outer quantiles located at 0.05 and 0.95 and the others equally spaced on the quantile scale (27.5^{th}, 50^{th}, and 72.5^{th} percentiles). The relative hazards of incident IS (with FBG of 100 mg/dL as reference) were graphed, first stratified by race/sex category and then by sex category to visualize the nature of the relationship between FBG and IS relationship in all demographic subgroups.

To account for MHT as a potential confounding variable, we then restricted our analysis to women and adjusted our model for ever vs. never use of MHT, in addition to the Framingham stroke risk factors, BMI, use of diabetes medications (no diabetes medication, oral diabetes medication, insulin use), education level, and household income. Finally, acknowledging that the association between FBG and IS may also differ between women who have never used MHT and those that have (i.e., effect modification), we restricted our analysis to women who reported never using MHT and repeated the analysis. P-values for the interaction terms for race by FBG were reported.

Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc, Cary, NC) and R Studio, version 3.6.1.[48]

Results

In total, 20,338 participants (200,586 person years) were included in our analyses with a median follow-up time of 11.4 years and 954 IS events (Figure 2.1). The mean age of participants was 64.5 (SD 9.3) years; 38.7% of participants were Black, 55.4% were women, and 16.2% were using DM medications at baseline (Table 2.1). The proportion of Black participants increased with increasing FBG level; 34.9% of participants with FBG < 100 were Black

compared with 63.6% of participants with FBG ≥ 200 mg/dL. The distribution of income and education levels also varied across FBG levels (Table 2.1). For example, of those with FBG <100, 14.2% were in the <$20,000 income bracket, while 29.3% of those in the ≥200 mg/dL category were in this lowest income bracket. With regard to the prevalence of hyperglycemia in our sample within race/sex subgroups, 7.7% of Black women (n=380), 8.6% of Black men (n=253), 3.3% of white women (n=206), and 4.5% of white men (n=278) had FBG levels ≥ 150.

Estimates for hazard ratios of IS associated with FBG, both pooled and sex/race specific, are displayed in Table 2.2 and Figure 2.2. Overall, compared to FBG < 100 mg/dL, FBG ≥ 150 mg/dL was associated with 59% higher hazards of IS (95% CI 1.21-2.08) and 61% higher hazards of IS when only those on diabetes medications were included (95% CI 1.12-2.31). In the full sample (Model 1), the association between FBG and IS varied by race/sex (HR for FBG ≥ 150 mg/dl compared to FBG <100 mg/dL: white women 2.05 (95% CI 1.23-3.42), Black women 1.71 (95%CI 1.10-2.66), Black men 1.24 (95%CI 0.75-2.06), white men 1.46 (95%CI 0.93-2.28), $p_{interaction}$=0.004). The race/sex differences were also present when restricting to participants on medications for diabetes at baseline (Model 2). The adjusted hazard ratio of IS for FBG ≥ 150 compared to FBG <100 was 3.30 (95% CI 1.20-9.10) for WW, 2.02 (95%CI 1.06-3.87) for BW, 1.24 (95%CI 0.63-2.46) for BM, and 1.08 (95%CI 0.53-2.17) for WM, $p_{interaction}$ =0.08. Among those not on diabetes medications (Model 3), IS risk across FBG did not vary significantly by race and sex (p=0.36).

Appendix Table A5 displays the results of analyses restricted to women only. From Model 4, adjusted for history of ever using MHT in addition to all other covariates, WW with FBG ≥ 150 mg/dL had 1.84 times the hazard of IS (95%CI 1.06-3.17) compared to those with FBG < 100, slightly higher than for BW (HR 1.54, 95%CI 0.96-2.48) and slightly attenuated

from effect estimates in Models 1 and 2. Findings from Model 5, restricting the sample to only women reporting no previous use of MHT, are also displayed in Appendix Table A3.

Figure 2.3 displays the hazard of IS by sex/race subgroup (Panel A) and by sex subgroup (Panel B) among participants using medications for diabetes, with FBG as a restricted cubic spline term rather than as a categorical variable. This figure demonstrates a difference in the association between FBG and IS by sex (p-value 0.03), with a higher HR for women than men across all FBG levels, but no significant interaction term between race/sex and FBG (p=0.20).

Discussion

Our study investigating the sex and race-specific risks of incident IS across increasing levels of FBG, a key glycemic maker in the diagnosis and management of DM, demonstrates that the pattern of IS risk with increasing FBG varies by sex, with no clear pattern by race. These findings add to the current knowledge of how previously observed sex differences in diabetes-associated stroke risk are affected by the control and severity of disordered glucose metabolism.[46] In our primary analysis, white women had the strongest association between increasing FBG and incident stroke, followed closely by Black women. Men, both white and Black, had a less predictable relation between increasing FBG and stroke risk. These sex and race differences in stroke risk by FBG appeared to be driven by participants who reported taking medications for DM and less so by those participants who either did not have diabetes or did not require medications for diabetes. When FBG was treated as a continuous variable and allowed to vary non-linearly with stroke risk, there was a J-shaped relationship between FBG and IS risk among women. This was not demonstrated in men.

Our findings of a higher stroke risk associated with increasing FBG in women compared with men point to possible mechanistic differences in the pathophysiologic process by which

clinical diabetes is related to cerebrovascular disease. Similar to prior literature demonstrating that sex differences in the stroke risk associated with diabetes were not explained by other vascular risk factors such as hypertension and obesity,[40] our findings remained despite adjustment for all the Framingham stroke risk factors along with obesity, income, and education. Further research is needed to investigate sex-specific contributors to vascular dysfunction related to disordered glucose metabolism such as insulin resistance, inflammation, coagulation, and the role of sex hormones. We were able to demonstrate that effect estimates for stroke risk were slightly attenuated when we adjusted for use of MHT and remained significant in white women when only those who had never used MHT were included, but we were not able to account for other hormone-related factors (i.e., history of pre-eclampsia) that may affect circulating active sex hormones and in turn, participants' vascular risk profiles (i.e., hyperandrogenism).

Although the magnitude of the association between FBG and stroke risk was higher for both Black and white women than their male counterparts, whether there were true differences in the strength of association between FBG and stroke between Black and white participants is not clear. There were, however, differences in the distribution of FBG levels between Black and white participants, suggesting potential disparities in the control of diabetes by race. Further investigation of diabetes control by race is needed in order to understand the potential relationship with BMI as well as social determinants of health (i.e., lower income, access to healthy foods) that are more common in Black participants and may directly result from inequities present in the healthcare system. Our data is consistent with previous data demonstrating poorer glycemic control among underrepresented minorities with DM[49,50] along with higher stroke incidence and mortality in Blacks compared with whites.[51]

The relation between FBG levels below 100 mg/dL and stroke risk is also somewhat unclear, though the J-shaped relationship we demonstrated among white women is consistent with prior data demonstrating a non-linear relationship between increasing FBG and vascular disease, defined by a composite outcome of coronary heart disease and ischemic stroke.[45] Our findings are also similar to prior data showing a significant association between FBG and vascular disease among persons with diabetes but not those without diabetes.[45] In our analysis, we chose to include participants not on diabetes medications in order to assess risk by sex and race across the full spectrum of fasting glucose, including those with undiagnosed diabetes and/or pre-diabetes not yet on medications, though the analysis of participants not on diabetes medications was limited due to small frequencies of stroke events.

Our findings have clinical implications related to the management of diabetes and suggest the need for future studies to further explore such sex differences. The sex differences in stroke risk in our study not only point to the need for strict adherence to current guidelines for stroke prevention among those with diabetes but also to the need for prospective studies to determine whether following sex-specific treatment guidelines for people with diabetes would reduce stroke incidence. In addition, it is possible that diabetes should be more heavily weighted for women than men in clinical risk scores for stroke. Finally, our findings point to the need for increased attention to management of DM among Blacks, who we know have higher stroke incidence and mortality from stroke.[51]

Several limitations need to be kept in mind when interpreting these findings. First, our primary analysis used baseline FBG; it is also important to consider other glycemic markers (e.g., hemoglobin A1C or insulin resistance) that are more reflective of long-term glucose control and/or better approximate the body's underlying ability to respond to insulin. Better measure

would only strengthen the findings. Second, although REGARDS has so far accumulated >200,000 person-years of follow-up, relatively small numbers of incident events occurred among some subgroups defined by race, sex, and FBG categories. Finally, we did not have data on other vascular risk factors that may affect both the degree of disordered metabolism and stroke risk in women; these include pre-eclampsia, gestational diabetes, and markers of hyperandrogenism.

Conclusions

Our data demonstrated sex differences in the association between impaired glucose metabolism and risk of IS, adding to existing knowledge that sex-specific stroke risk associated with DM is higher among women than men. Further research is needed to identify how such differences might be incorporated into clinical care guidelines. Finally, disparities in glycemic control by race point to the need for further intervention to prevent stroke in racial minorities.

Tables and Figures

Table 2.1 Baseline characteristics

		Fasting blood glucose category (mg/dL)				
	Total	**<100**	**100-125**	**126-149**	**150-199**	**≥200**
Overall, N (%)	20,338	13547 (66.6)	4707 (23.1)	967 (4.8)	735 (3.6)	382 (1.9)
Characteristics						
Sex, N(%)						
Female	11263 (55.4)	7768 (57.3)	2411 (51.2)	498 (51.5)	384 (52.2)	202 (52.9)
Race, N (%)						
Black	7880 (38.7)	4734 (34.9)	2009 (42.7)	504 (52.1)	390 (53.1)	243 (63.6)
White	12458 (61.3)	8813 (65.1)	2698 (57.3)	463 (47.9)	345 (46.9)	139 (36.4)
Age, Mean (SD)	64.5 (9.3)	64.3 (9.5)	64.9 (9.0)	65.7 (8.8)	64.4 (8.5)	62.7 (8.5)
Education, N (%)						
< High school	2190 (10.8)	1250 (9.2)	538 (11.4)	172 (17.8)	142 (19.3)	88 (23.0)
High school	5176 (25.4)	3325 (24.5)	1270 (27.0)	269 (27.8)	210 (28.6)	102 (26.7)
Some college	5471 (26.9)	3667 (27.1)	1251 (26.6)	260 (26.9)	190 (25.9)	103 (27.0)
≥ College graduate	7501 (36.9)	5305 (39.2)	1648 (35)	266 (27.5)	193 (26.3)	89 (23.3)
Income, N (%)						
Less than $20k	3216 (15.8)	1924 (14.2)	782 (16.6)	225 (23.3)	173 (23.5)	112 (29.3)
$20k-$34k	4865 (23.9)	3182 (23.5)	1111 (23.6)	275 (28.4)	201 (27.3)	96 (25.1)
$35k-$74k	6356 (31.3)	4305 (31.8)	1511 (32.1)	250 (25.9)	187 (25.4)	103 (27.0)
≥ $75k	3540 (17.4)	2527 (18.7)	777 (16.5)	118 (12.2)	81 (11.0)	37 (9.7)
Refused	2361 (11.6)	1609 (11.9)	526 (11.2)	99 (10.2)	93 (12.7)	34 (8.9)
BMI, Mean (SD)	29.1 (6.1)	28.0 (5.6)	30.7 (6.1)	32.5 (6.5)	33.1 (6.7)	32.3 (6.4)
SBP, Mean (SD)	126.9 (16.4)	125.1 (16.1)	129.4 (16.1)	132.0 (17.1)	132.9 (17.0)	133.4 (17.5)
Use of blood pressure medications, N(%)	10198 (50.1)	5970 (44.1)	2770 (58.8)	673 (69.6)	530 (72.1)	255 (66.8)
LVH, N (%)	1851 (9.1)	1094 (8.1)	484 (10.3)	130 (13.4)	89 (12.1)	54 (14.1)
Atrial fibrillation, N (%)	1540 (7.6)	971 (7.2)	376 (8.0)	91 (9.4)	66 (9.0)	36 (9.4)
History of heart disease, N (%)	3225 (15.9)	1889 (13.9)	826 (17.5)	230 (23.8)	177 (24.1)	103 (27.0)
Smoking status, N (%)						
Current	2831 (13.9)	1837 (13.6)	687 (14.6)	146 (15.1)	98 (13.3)	63 (16.5)
Past	8103 (39.8)	5187 (38.3)	2028 (43.1)	423 (43.7)	301 (41.0)	164 (42.9)
Never	9404 (46.2)	6523 (48.2)	1992 (42.3)	398 (41.2)	336 (45.7)	155 (40.6)
	3842 (18.9)	779 (5.8)	979 (20.8)	967 (100)	735 (100)	382 (100)

Documented history of diabetes at baseline, N (%)						
Ever MHT Use,* N (%)	6363 (56.7)	4488 (58.0)	1369 (56.9)	249 (50.2)	168 (43.9)	89 (44.5)

SD: standard deviation, BMI: body mass index, SBP: systolic blood pressure, LVH: Left ventricular hypertrophy, MHT: menopausal hormone therapy; Sample size for Ever MHT Use = 11224

Table 2.2: Estimates for Risk of Ischemic Stroke Associated with Increasing Fasting Blood Glucose by Race/ Sex Group

	Model 1: All Participants (N= 20338)		**Model 2: Participants on Diabetes Medications (N=3293)**		**Model 3: Participants Not on Diabetes Medications (N=17045)**	
Fasting Blood Glucose	**Stroke events/ N**	**Adjusted Hazard Ratios (95% CI)**	**Stroke events/ N**	**Adjusted Hazard Ratios (95% CI)**	**Stroke events/ N**	**Adjusted Hazard Ratios (95% CI)**
<100 (Ref)						
BW	110/3004	1.0	14/291	1.0	96/2713	1.0
WW	167/4764	1.0†	5/118	1.0†	162/4646	1.0
BM	84/1730	1.0	15/191	1.0	69/1539	1.0
WM	194/4049	1.0	15/179	1.0	179/3870	1.0
Overall HR		1.0†		1.0		1.0
100-125						
BW	60/1252	1.17 (0.85, 1.60)	15/323	0.94 (0.45, 1.95)	45/929	1.32 (0.93, 1.89)
WW	62/1159	1.39 (1.04, 1.87)	19/187	2.66 (0.98, 7.18)	43/972	1.21 (0.86, 1.70)
BM	45/757	1.18 (0.82, 1.71)	16/199	1.06 (0.52, 2.15)	29/558	1.18 (0.76, 1.82)
WM	82/1539	1.00 (0.77, 1.30)	14/270	0.60 (0.29, 1.24)	68/1269	1.10 (0.83, 1.45)
Overall HR		1.17 (1.00, 1.37)		1.03 (0.71, 1.50)		1.19 (1.00, 1.41)
126-149						
BW	17/298	1.23 (0.73, 2.08)	13/195	1.38 (0.65, 2.94)	4/103	1.20 (0.44, 3.27)
WW	24/200	2.72 (1.74, 4.25)	17/115	4.17 (1.52, 11.42)	7/85	2.26 (1.06, 4.84)
BM	10/206	0.80 (0.41, 1.56)	6/138	0.56 (0.22, 1.45)	4/68	1.21 (0.44, 3.32)
WM	8/263	0.45 (0.22, 0.93)	7/171	0.48 (0.20, 1.19)	1/92	0.23 (0.03, 1.67)
Overall HR		1.14 (0.85, 1.53)		1.13 (0.75, 1.70)		1.14 (0.69, 1.88)
≥150						
BW	29/380	1.71 (1.10, 2.66)	28/338	2.02 (1.06, 3.87)	1/42	0.93 (0.13, 6.68)
WW	18/206	2.05 (1.23, 3.42)	17/159	3.30 (1.20, 9.10)	1/47	0.59 (0.08, 4.23)
BM	20/253	1.24 (0.75, 2.06)	19/206	1.24 (0.63, 2.46)	1/47	0.46 (0.06, 3.29)
WM	24/278	1.46 (0.93, 2.28)	17/213	1.08 (0.53, 2.17)	7/65	2.47 (1.16, 5.28)
Overall HR		1.59 (1.21, 2.08)		1.61 (1.12, 2.31)		1.29 (0.69, 2.42)
P-value for race/sex by FBG interaction term		0.0036*		0.08*		0.36

BW: Black women, WW: White women, BM: Black men, WM: White men
Models are adjusted for age, age by race/sex, FBG, race/sex by FBG, body mass index, systolic blood pressure, use of anti-hypertensive medications, left ventricular hypertrophy, atrial fibrillation, history of coronary artery disease, smoking, education, income
*Prespecified $P < 0.10$ considered statistically significant for interaction terms, †P-value <0.05 for linear trend

Figure 2.1: Flowchart of Study Sample

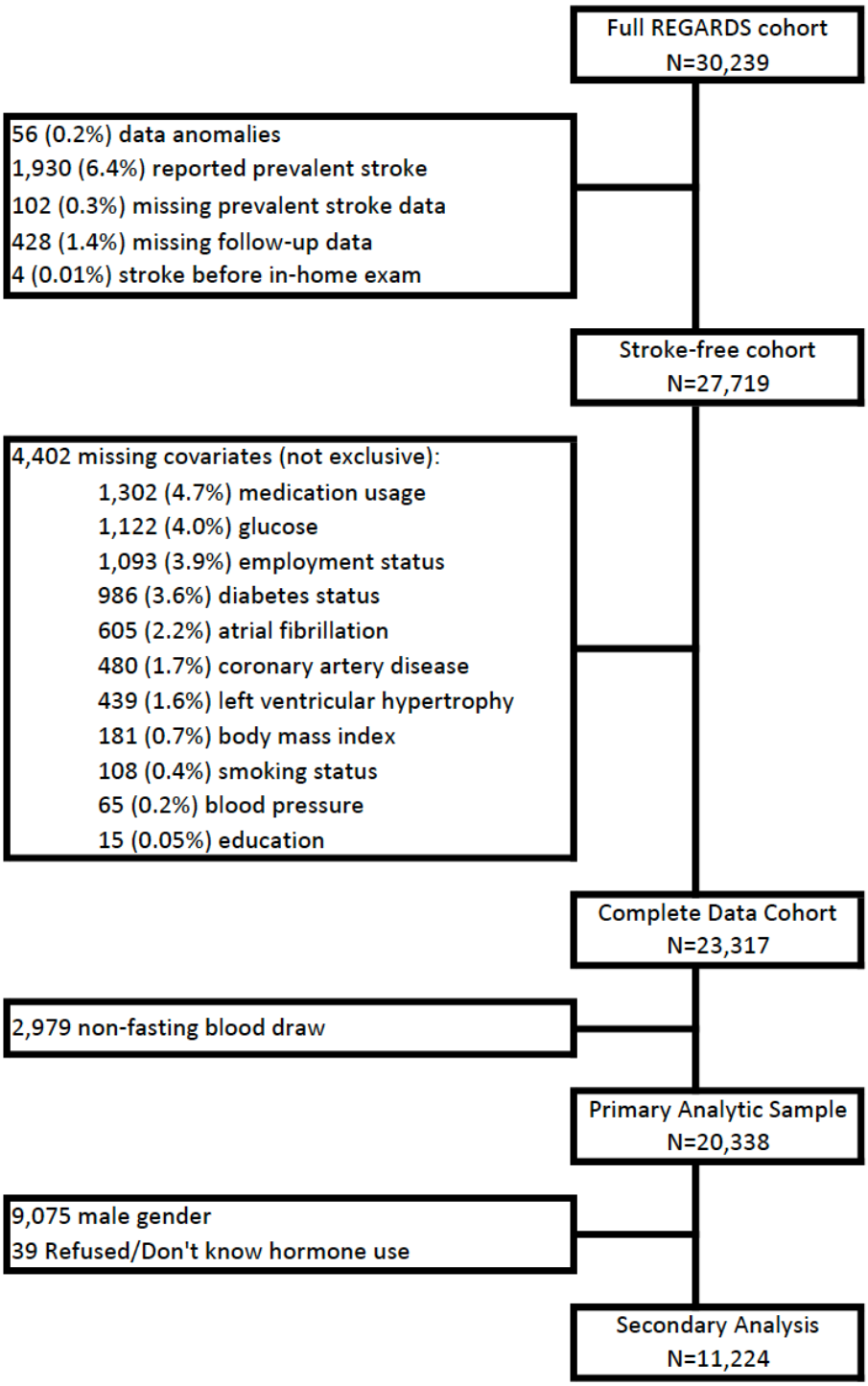
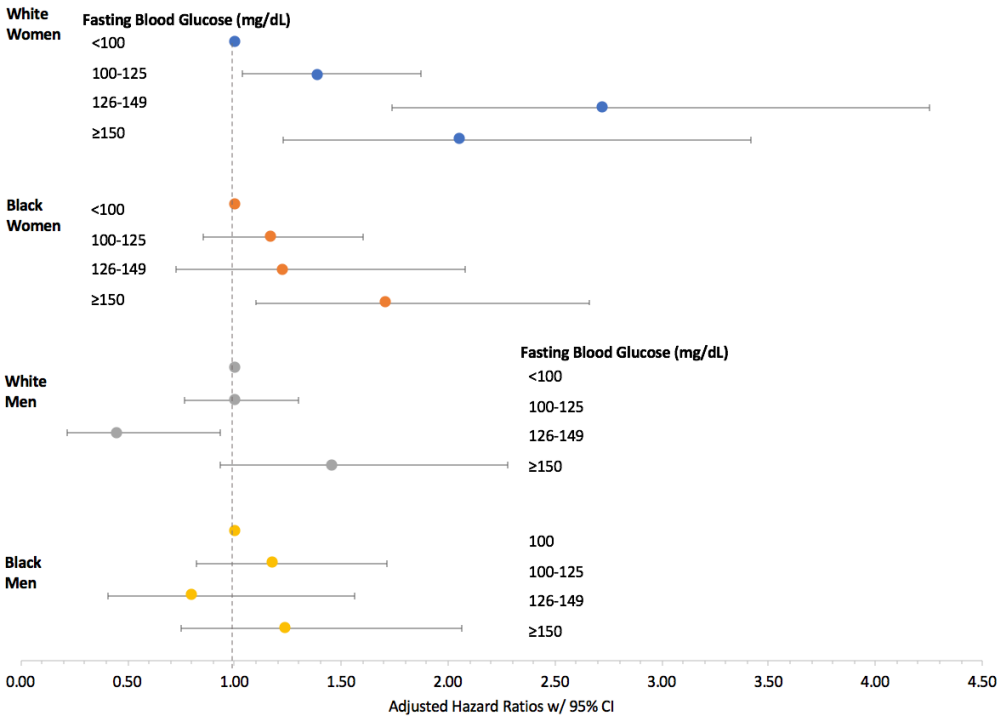


Figure 2.2: Adjusted Relative Hazards of Incident Ischemic Stroke by Fasting Blood Glucose Level and Sex/Race Subgroups, Adjusted by Use of Diabetes Medications

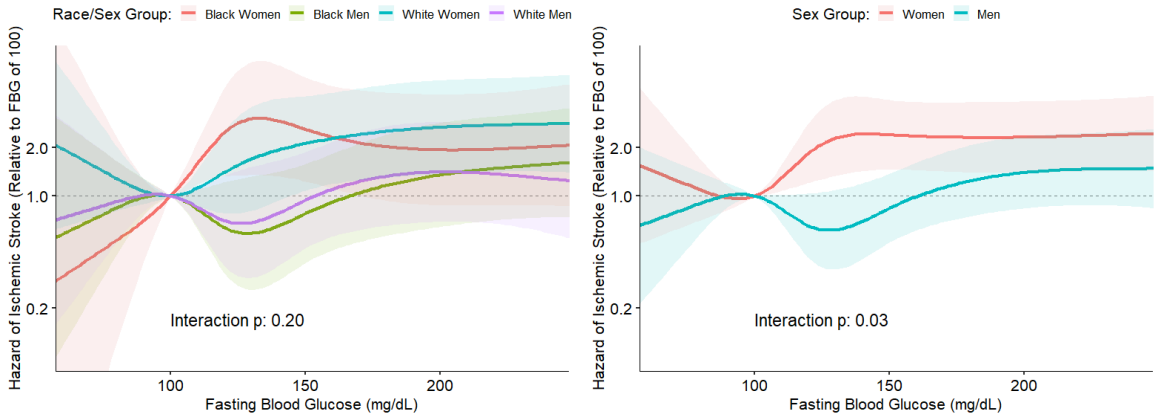


Adjusted for age, age*race/sex, race/sex, medication use (no diabetes medication, oral diabetes medication, insulin use), BMI, SBP, use of anti-hypertensive medications, LVH, atrial fibrillation, history of CAD, & smoking, education, income

Figure 2.3: Relative Hazard of Ischemic Stroke by Fasting Blood Glucose as a Spline Among Participants on Diabetes Medications

Panel A: By race/sex category

Panel B: By sex category



Panels A and B display relative hazards of ischemic stroke by fasting blood glucose (as restricted cubic spine term with 5 knots), adjusted for age, race, and race by age interaction term

CHAPTER 3: Evidence for the Critical Role of Sex Hormone Binding. Globulin (SHBG) in the development of Ischemic Stroke

Abstract

Circulating levels of sex hormone binding globulin (SHBG) have been inversely linked to obesity, diabetes, and other cardiometabolic disorders. It remains uncertain whether low SHBG is prospectively predictive of stroke risk, particularly in women. We investigated whether SHBG is associated with risk of incident ischemic stroke (IS) among women in the Women's Health Initiative (WHI). From an observational cohort of 161,808 postmenopausal women enrolled in the WHI at 40 sites across the U.S. from 1993 – 1998, we identified 13,192 participants free of prevalent stroke at baseline who were included in an ancillary study that measured serum SHBG. We used Cox proportional hazards regression, stratified by SHBG measurement assay, to assess IS risk across quintiles of SHBG (Q1 – Q5), adjusting first for demographic variables (Model 1), additionally for body mass index, hypertension, alcohol use, and smoking status (Model 2), and for physical activity and reproductive risk factors (Model 3). In sensitivity analyses, potential mediators (diabetes status, levels of estradiol, testosterone, and CRP) were included. Of 13,192 participants (mean age 62.5 years, 67.4% non-Hispanic white, 18.5% Black, 7.6% Hispanic, 5.0% Asian), after following for an average of 11.6 years, 768 IS events were adjudicated. Compared to the highest quintile of SHBG levels (referent), women in the lowest SHBG quintile had a higher risk of IS in all three multivariable-models (Model 1: HR 1.88, 95%CI 1.47-2.41, Model 2: HR 1.69, 95%CI 1.30-2.20, Model 3: HR: 1.61, 95%CI 1.19-2.19, trend tests $p < 0.05$ for all models). Including potential mediators such as diabetes, estradiol, and testosterone in the models attenuated but did not eliminate significant inverse associations between SHBG and IS. In this prospective cohort of post-menopausal women, there was a

statistically significant inverse association between serum SHBG levels and IS risk, which supports the notion that SHBG could be used as a risk stratification tool for predicting IS in women.

Introduction

Age-adjusted risk of ischemic stroke (IS) is generally thought to be lower among women than men;[10] however, incidence rates between women and men become similar among older age groups,[10] with rates in women over 80 years of age surpassing those of men in some studies.[22,52,53] Due to longer life expectancies, women, overall, have a higher lifetime risk of stroke compared with men.[1] Sex differences in the risk of IS across the life course may be related to protective effects of endogenous sex steroids such as estradiol in pre-menopausal women and the lack of these effects among older women,[54] though trials of exogenous hormones for the prevention of stroke in post-menopausal women demonstrated an increased risk of stroke with the use of both estrogen and estrogen plus progestin. These increased risks contributed to the termination of the Women's Health Initiative (WHI) hormone therapy trials.[55]

A gap exists in understanding the role of endogenous hormones in IS risk, especially among post-menopausal women. Data on endogenous estradiol and risk of cardiovascular disease and/or stroke among women conflict,[56,57] though some studies have reported increased stroke risk with higher estradiol levels.[57] This presents a need to continue to study the role of sex hormones and their related proteins. Sex hormone-binding globulin (SHBG), a protein that binds to and regulates available testosterone and estradiol, is one potential target. Previous literature has demonstrated inverse associations between SHBG and vascular risk factors (i.e., insulin resistance, inflammation, diabetes, and metabolic syndrome) as well as outcomes, specifically coronary heart disease (CHD) in some studies.[56,58–64] The mechanism by which low SHBG levels are related to an increased risk of vascular disease and outcomes is not well understood but likely

includes a combination of indirect effects through alterations in the balance between testosterone and estradiol as well direct effects, independent of sex steroids.[65]

Despite data linking SHBG to vascular risk factors and outcomes such as diabetes and CHD, it remains uncertain whether SHBG is associated with stroke risk, particularly in women. In the current study, our objective was to investigate the association between SHBG and incident IS among post-menopausal women using a subsample from the WHI study.

Methods

Study Population/ Study Design

The WHI is a large, national, prospective cohort study that comprises a group of randomized clinical trials and an observational study. Between 1993 and 1998, 161,808 postmenopausal women, ages 50-79 years at baseline, were enrolled at one of 40 clinical sites in the United States. Follow-up for the main study was through 2005, at which time participants were asked to reconsent to further follow-up through 2010, with a second reconsenting process occurring at that time. Participants had a baseline study visit between 1993 and 1998 followed by annual visits and semi-annual contacts.

Our study sample (Figure 3.1) is comprised of participants in one of eleven ancillary case control studies in which SHBG was measured as part of the study protocol (Appendix Table A6). Participants were excluded from the analysis if they had a history of any strokes at baseline or if they had missing data on any of the key model covariates. Use of the WHI data for this analysis was approved by the institutional review boards of each principal investigator's institution.

Exposure Measurement

The primary exposure variable was serum SHBG at the time of enrollment. Blood samples were drawn at the initial study visit and stored at -80 degree Celsius. Serum SHBG concentration in nanomoles per liter (nmol/L) was measured at one of three labs (each

performing a single type of assay): an electrochemiluminescence immunoassay (Roche Diagnostics) for ancillary study 238 (12.7% of participants), an immunoradiometric assay (Esoterix) for ancillary study W5 (2.2% of participants), and a two-site chemiluminescent immunoassay (Siemens Medical Solutions) for the remaining 9 studies (85.1% of participants). Data on SHBG levels using participants in this group of ancillary studies have been pooled previously, and interassay coefficients of variation between 3.7% and 17.7% were reported.[66]

Outcome

The outcome of interest was incident IS during the follow-up period. Potential stroke events were initially identified at the time of semi-annual contacts or annual visits using medical history update forms completed by participants. All potential stroke events were then adjudicated by trained study physicians using medical records comprised of physician notes, diagnosis codes, and imaging results. Stroke was defined as the rapid onset of a persistent neurologic deficit lasting at least 24 hours due to cerebrovascular obstruction. To be included as an event, the relevant deficit must have lasted at least 24 hours or have a compatible lesion on CT and/or MRI. Events were considered ischemic (rather than primary hemorrhagic or unknown) if: 1) there was a focal deficit without blood on CT, MRI, or LP (if performed); 2) if brain imaging (CT or MRI) showed hypodensities in a tissue pattern compatible with the symptoms; or 3) if there was surgical or autopsy evidence of infarct. Given prior evidence linking SHBG to risk factors for ischemia (i.e., diabetes and insulin resistance) as well as CHD, the focus of this study was on ischemic cerebrovascular events; participants with primary hemorrhage were not included due to concerns for significant differences in pathophysiology and risk factor profiles between stroke subtypes.

Covariates

All covariates included in the analysis were collected at the time of study enrollment. Age, race/ethnicity, and medical comorbidities including history of hypertension and history of ever being treated for diabetes were collected via interview. Social history including alcohol use (≤ 1 drink/ month, 1-7 drinks per week, and ≥ 7 drinks per week) and smoking status (never/ past/ current) were also collected at the time of enrollment via self-report. Body mass index (BMI) (weight in kilograms / (height in meters)^2) was collected using baseline height and weight measured at enrollment, and physical activity was reported in met-hours per week. Physical activity, defined as total weekly energy expended through recreational activity, was calculated by combining questions in which women reported frequency and duration of weekly walking, mild, moderate, and strenuous physical activity.

Additionally, reproductive risk factors including age at menarche, age at menopause (as defined as ≥ 1 year without regular periods), history of ever using oral contraceptives, number of full-term pregnancies (≥6 months gestational age), and use of menopausal hormone therapy (MHT) (never/past/ current) were collected via self-report at the time of enrollment. MHT was defined as the use of unopposed estrogen or estrogen plus progesterone through a pill or patch.

Biomarker covariates for secondary analyses

Total serum estradiol and total testosterone were measured at baseline visits as part of the ancillary studies described above. Serum C-reactive protein (CRP) was measured at baseline on a sample of participants who were included in the WHI CVI Biomarkers Study. Further detail on sampling schemes and measurement methods can be found on the WHI website.[67]

Statistical Analysis

To understand the nature of the relationship between SHBG and incident IS, we analyzed SHBG in two ways: first in categories (quintiles) and also as a log-transformed continuous

variable due to the right-skewed distribution of the variable. Baseline characteristics of included participants were described using frequencies and proportions for categorical variables and means and standard deviations (SD) (or medians and interquartile ranges (IQR)) for continuous variables as appropriate, in the overall sample and by SHBG quintile.

We conducted time to event analyses. Participants that did not have an IS during the follow-up period (within 15 years from time of enrollment) were censored due to death, loss to follow-up, or administrative censoring, and length of follow-up in days for each participant is pre-calculated in the WHI dataset. Those participants who were still being followed at 15 years were administratively censored to ensure a standardized length of follow-up across studies.

The association between SHBG and incident IS was first investigated using Kaplan-Meier curves with log rank tests for statistical significance. Next, we used Cox proportional hazards regression to investigate the association of SHBG with IS in sequential models with the highest quintile (Q5) as the referent group, first adjusted for demographics (age, race/ethnicity). Stratification by SHBG measurement assay (with the 'strata' statement in SAS) was performed to reduce possible biases relating to the combination of 3 different SHBG assays as described above. Next, we additionally adjusted for BMI, history of hypertension, history of alcohol use, and smoking status. The final model in the sequence was also adjusted for physical activity and risk factors related to participants' reproductive history (age at menarche, age at menopause, number of full-term pregnancies, history of use of OCPs, and history of MHT use). All potential confounders were chosen based on prior literature or subject knowledge suggesting a potential causal relationship with both the exposure (SHBG) and the outcome (stroke). See Figure 3.2 for a directed acyclic graph of the proposed relationship between SHBG and IS.

Similar sequential Cox proportional hazards regression models were performed with log-transformed SHBG rather than SHBG in quintiles to assess the robustness of our findings and to account for lack of clarity around the appropriate cut-off value of SHBG.

The proportionality assumption was assessed using visualization of Schoenfeld residuals and a test of proportionality when a time x SHBG term was included in each model. For all Cox proportional hazard models, hazard ratios and the corresponding 95% confidence intervals were reported. Statistical significance was assessed using an alpha level of 0.05 (two-sided).

Sensitivity analyses

In a pre-planned sensitivity analysis, models were adjusted for whether participants were a case (vs. control) in any of the ancillary studies to adjust for potential selection bias. Additionally, analyzing SHBG as a log-transformed continuous variable, we stratified Cox models by individual ancillary study to account for potential bias introduced by combining studies. Stratification by ancillary study was not performed with SHBG as quintiles due to very small numbers of stroke events by quintile in individual ancillary studies. Our final sensitivity analysis was adding history of liver disease to the previously adjusted models, given that SHBG Is primarily produced by the liver.

Exploratory Analyses with Potential Mediators

To identify potential mediators in the relationship between SHBG and IS, a variable for each potential mediator was added into the models one at time (along with an SHBG x mediator product term) to evaluate whether inclusion in the models reduced the inverse association between SHBG and IS risk. Only those participants who had valid measurements for mediators were included in each of these models, and due to reduced sample sizes, Cox models were not stratified by SHBG assay with the exception of the DM mediation model which included almost the full sample. The potential mediators included history of diabetes, total serum estradiol and

testosterone levels, and C-reactive protein. Once again, hazard ratios with 95% confidence intervals were reported.

All statistical analyses were performed using SAS version 9.4.

Results

Our primary analysis included 13192 unique participants (Figure 3.1) with 768 IS events. In the entire sample, the mean follow-up time was 11.6 years. Of the total sample, 3.1% were lost to follow-up, 27.5% were censored due to death, and 63.6% were administratively censored.

Baseline characteristics of the participants by SHBG quintiles are displayed in Table 3.1. The median age of participants ranged between 63.0 (IQR 58.0-69.0) and 67.0 (IQR 61.0-72.0). The age distribution varied by SHBG level ($p<0.0001$ for Wilcoxon rank sum test across quintiles): participants in the lowest quintile were younger than those in the other quintiles. Non-Hispanic white participants comprised 67.4% of the sample, 18.5% of the overall participants were Black, 7.6% were Hispanic, and 5.0% were Asian. There were no clear trends in race/ethnicity distribution across SHBG levels.

Risk factor profiles differed across SHBG quintiles. Compared with participants in the highest SHBG quintile, those in the lowest SHBG quintile had a higher mean BMI (32.2(SD 6.0) vs. 26.2(SD 5.5), respectively $p<0.0001$) and had higher proportions of hypertension (48.8% vs. 32.5%, respectively, $p<0.0001$) and diabetes at baseline (11.7% vs. 2.4%, respectively, $p<0.0001$). With respect to reproductive risk factors, mean age at menopause was similar across SHBG quintiles. Compared with those in the highest quintile, those in the lowest quintile of SHBG were more likely to report current use of MHT (4.2% vs. 21.4%, respectively, $p<0.0001$).

Unadjusted, a greater proportion of participants in the lowest quintile had IS (6.9%) compared with the highest quintile (3.8%), $p<0.0001$. Accounting for length of follow-up time using Kaplan-Meier curves with log-rank tests, results were similar ($p<0.0001$, Figure 3.3).

Results of sequential Cox proportional hazard models are displayed in Table 3.2 and Figure 3.4. Stratified by SHBG assay and adjusted for age and race, those in the lowest quintile (Q1) of SHBG had 88% higher hazards (95% CI 1.47-2.41) of IS compared with those in the highest reference group (Q5) (trend test $p<0.0001$). Adjustment for BMI, history of hypertension, alcohol use, and smoking status resulted in somewhat attenuated hazard ratios (Q1 vs Q5 (ref), 1.69, 95% CI 1.30-2.20, trend test $p=0.004$). The addition of weekly reported physical activity and reproductive risk factors (age at menopause, number of full term pregnancies, MHT use at baseline, history of OC use, and age at menarche) resulted in a HR of 1.61, 95%CI 1.19-2.19, trend test $p=0.04$). Hazard ratios for Q2, Q3, and Q4 were smaller but still demonstrate an inverse association with IS risk compared with Q5 (highest quintile and reference group) (Table 3.2).

When log-transformed and treated as a continuous variable, a one-unit increase in log-SHBG was associated with an inverse association with IS (HR 0.77 (95%CI 0.67-89), adjusted for age, race/ethnicity, BMI, history of hypertension, alcohol use, smoking, and stratified by SHBG assay.

Sensitivity Analyses

Next, we adjusted for whether participants were a case in any one of the ancillary studies to account for potential selection bias; effect estimates were similar to previous models (Q1 vs. Q5, HR 1.74 (95%CI 1.34-2.25), adjusted for age, race/ethnicity, BMI, history of hypertension, alcohol use, and smoking status.

Additionally, stratifying by individual ancillary study, and adjusting for age, race/ethnicity, BMI, history of hypertension, alcohol, and smoking, the HR for IS per unit increase in log SHBG was 0.86 (0.74-0.99). The small number of stroke events (<10) in some of the individual ancillary studies, however, limit the interpretability of this result.

Finally, adding history of liver disease to the models did not change our effect estimates (Appendix Table A7).

Results of Secondary Analyses

Finally, in subsets of the sample, Table 3.3 demonstrates results of our exploratory analyses of potential mediators in the relationship between SHBG and incident IS. The diabetes, estradiol/ testosterone, and CRP models demonstrated attenuation of the effect estimates compared with hazard ratios in Table 3.2. Interaction terms for each of the potential mediators (history of diabetes x SHBG, estradiol level x SHBG, testosterone level x SHBG, and CRP level x SHBG) were all non-significant ($p>0.05$).

Discussion

In this prospective cohort of post-menopausal women, there was a statistically significant inverse association between SHBG level and IS risk. Those in the lowest SHBG quintile had less favorable cardiometabolic profiles compared with participants in higher quintiles, and the risk of IS was between 1.6 and 1.9 times higher among those in the lowest SHBG quintile compared with those in the highest SHBG quintile. Though these findings are novel with respect to the evaluation of stroke as the primary outcome, our findings are supported by studies showing an inverse association between SHBG and cardiovascular disease.[56,68] Further, while some previous prospective studies of SHBG and CVD outcomes have reported that the association is almost completely explained by other risk factors such as BMI,[56] our findings of an inverse association

between SHBG level and IS risk were robust to adjustment for demographic factors, BMI, hypertension, smoking, alcohol, and reproductive risk factors including age at menopause and history of MHT use. Other previous data have demonstrated that low SHBG levels are linked to greater odds of having significant carotid atherosclerosis, which may point to a possible mechanism between low SHBG and stroke.[69] Though our findings help to establish an epidemiologic link between SHBG and IS, it is important to consider that our data cannot demonstrate biologic plausibility. Though purely speculative, hypotheses for the biologic mechanisms include factors such as large vessel atherosclerosis or changes in thrombogenesis among those with embolic stroke.

With respect to the pattern of the relationship between SHBG and incident IS, the largest effect sizes were seen between the top and bottom quintiles (Q5 and Q1). The middle groups (Q2-Q4) all had hazards of stroke > 1 but did show a clear dose response relationship, suggesting the need for further work to identify a cutoff point for SHBG at which stroke risk may increase.

Though the current study was not designed to evaluate either a causal relationship or the biologic mechanism between low SHBG levels and IS, our secondary analyses of potential mediating variables provide some hypotheses-generating data about possible mechanisms. For example, the model that included diabetes as a covariate showed an attenuation of effect estimates, suggesting that some but not all of the association between low SHBG and stroke risk could be due to development of insulin resistance and clinical diabetes. This is in line with several previous studies demonstrating evidence for a causal relationship between low SHBG and both insulin resistance and clinical diabetes.[58,59,62] Other secondary analyses that included estradiol, testosterone, and CRP are limited by small sample sizes, but inclusion of testosterone and CRP in the models also attenuated effect estimates, supporting the possibility that some of

the relationship between low SHBG and IS may be related to either increasing free testosterone with downstream pro-androgenic effects[56] and/or inflammatory pathways.[60,70] These secondary analyses, however, are limited by data availability, smaller sample sizes, and a lack of dose response patterns; formal mediation analyses could be performed in future studies.

Our findings of the association between low SHBG and stroke risk have potential implications for the way in which we predict stroke risk in post-menopausal women. For example, the association between low SHBG and IS persisted despite adjustment for many of the classically defined stroke risk factors (i.e., hypertension), suggesting that a measure of endogenous sex hormones like SHBG could possibly improve the performance of commonly used prediction tools. Expert guidelines on the topic of stroke prevention have called for the need to incorporate sex-specific risk factors into current prediction tools;[12] in the future, this could include hormonal biomarkers as well as aspects of reproductive history. CHA_2DS_2-VASc is one tool that incorporates patient sex to better predict stroke in the setting of atrial fibrillation;[71] studying the addition of hormonal biomarkers to this rule for both sexes might be one potential future research direction. It is also unclear what an optimal SHBG value would be to use for clinical risk prediction; this could also be assessed in future studies.

Our findings also have potential implications for novel risk factor modification strategies. Not only might SHBG be used in the future as way to improve prediction of stroke risk, but it could serve as a therapeutic target. This is especially appealing given the association between low SHBG and overall poor cardiometabolic health.[64] Previous studies have shown positive associations between modifiable lifestyle factors like exercise,[66] diet[72] and SHBG; whether SHBG could be predictably modified by changes in lifestyle is unknown.

Strengths and Limitations

Our paper has several strengths and limitations that should be noted. A clear strength is the large sample size with racial and ethnic diversity along with the prospective study design of the WHI. There is, however, potential selection bias related to the combination of case control studies which may be present despite our statistical methods including adjustment for case vs. control status in the regression model. In addition, though we were able to adjust for a large number of potential confounding variables, our study was not designed to test for a causal relationship between low SHBG and stroke but only to test associations. We performed exploratory analyses to assess the effect of including potential mediators on the association between low SHBG and incident IS. Since data on exposure variables and mediators were both obtained at baseline, though, the temporal relationship between SHBG and potential mediators cannot be truly determined. Though cross sectional in nature, these secondary analyses could be used to guide future formal mediation analyses. Finally, our results may not be generalizable to hemorrhagic strokes.

Conclusions

In this prospective cohort of post-menopausal women, there was a significant inverse association between SHBG levels and IS risk, suggesting that that SHBG could improve risk stratification for predicting IS in post-menopausal women. Future research is needed on the nature of the relationship between SHBG and stroke (causal or not), the potential mechanisms, and the ability to use SHBG to improve our current methods of stroke prediction and prevention in women.

Tables and Figures

Table 3.1 Baseline Characteristics of Participants by Circulating Levels of Sex Hormone Binding Globulin Level

Baseline variables	**SHBG Q1 (n=2639)**	**SHBG Q2 (n=2601)**	**SHBG Q3 (n=2675)**	**SHBG Q4 (n=2639)**	**SHBG Q5 (n=2639)**	**p-value**
SHBG (Median (IQR))	23.3 (19.1-26.7)	35.3 (32.6-38.0)	47.0 (43.8-50.6)	64.0 (58.6-69.8)	107 (88.5-141.0)	n/a
Age (Median (IQR))	63.0 (58.0-69.0)	66.0 (60.0-71.0)	67.0 (60.0 – 72.0)	67.0 (61.0-72.0)	66.0 (59.0-71.0)	<0.0001
Race/ethnicity, N (%)						<0.0001
American Indian/Alaskan Native	36 (1.4)	24 (0.9)	27 (1.0)	15 (0.6)	15 (0.6)	
Asian/ Pacific Islander	123 (4.7)	99 (3.8)	118 (4.4)	117 (4.4)	193 (7.3)	
Black/ African-American	534 (20.2)	459 (17.6)	458 (17.1)	440 (16.7)	547 (20.7)	
Hispanic/Latino	216 (8.2)	203 (7.8)	173 (6.5)	168 (6.4)	240 (9.1)	
Non-Hispanic white	1712 (64.9)	1794 (69.0)	1879 (70.2)	1885 (71.4)	1620 (61.4)	
Other	18 (0.7)	22 (0.8)	20 (0.7)	14 (0.5)	24 (0.9)	
Body Mass Index (Mean (SD))	32.2 (6.0)	30.2 (5.9)	28.7 (5.9)	27.0 (5.6)	26.2 (5.5)	<0.0001
History of Hypertension, N (%)	1289 (48.84)	1080 (41.52)	1017 (38.02)	927 (35.13)	858 (32.51)	<.0001
History of Diabetes, N (%)	308 (11.7)	168 (6.5)	124 (4.6)	82 (3.1)	64 (2.4)	<0.0001
Physical Activity (Met-hours/week), Median (IQR)	4.5 (0.5-11.8)	6.0 (1.5-14.1)	7.5 (1.9-16.7)	8.3 (2.5-18.3)	8.3 (2.2-18.3)	<0.0001
Alcohol Use, N (%)						<0.0001
≤1 drink/month	1501 (56.9)	1309 (50.3)	1314 (49.1)	1243 (47.1)	1328 (50.3)	
1-7 drinks/ per week	922 (34.9)	1024 (39.4)	1076 (40.2)	1111 (42.1)	1082 (41.0)	
≥ 7 drinks/ week	216 (8.2)	268 (10.3)	285 (10.6)	285 (10.8)	229 (8.7)	
Smoking Status, N (%)						0.002
Never	1368 (51.84%)	1378 (52.98%)	1445 (54.02%)	1372 (51.99%)	1418 (53.73%)	
Past	1087 (41.19%)	1001 (38.49%)	1000 (37.38%)	1010 (38.27%)	968 (36.68%)	
Current	184 (6.97%)	222 (8.54%)	230 (8.60%)	257 (9.74%)	253 (9.59%)	
Age at menopause (Mean, SD)	47.8 (6.7)	47.9 (6.8)	48.0 (6.6)	48.5 (6.3)	48.1 (6.5)	0.004
Age at menarche, N (%)						0.001
≤ 10	97 (4.4)	97 (4.4)	88 (3.8)	65 (2.9)	84 (3.8)	
11	268 (12.2)	234 (10.7)	251 (11.0)	192 (8.9)	198 (9.0)	
12 (ref)	464 (21.1)	446 (20.4)	491 (21.4)	476 (21.5)	426 (19.3)	
13	541 (24.5)	582 (26.6)	565 (24.7)	568 (25.7)	570 (25.6)	

14 ≥ 15	348 (15.8) 486 (22.1)	374 (17.1) 458 (20.9)	388 (16.9) 508 (22.2)	414 (18.7) 497 (22.5)	385 (17.5) 540 (24.5)	
Oral contraceptive use ever, N (%)	1039 (39.4)	911 (35.0)	854 (31.9)	829 (31.4)	876 (33.2)	<.0001
Number of full-term pregnancies, N (%) 0 1 2-4 ≥5	 304 (11.6) 230 (8.8) 1550 (59.0) 541 (20.6)	 275 (10.6) 223 (8.6) 1542 (59.3) 559 (21.5)	 313 (11.8) 233 (8.9) 1615 (60.8) 494 (18.6)	 315 (12.0) 252 (9.6) 1604 (61.3) 445 (17.0)	 306 (11.7) 271 (10.3) 1590 (60.7) 452 (17.3)	0.0010
Use of MHT at baseline, N (%) Never Past Current	1904 (72.1) 623 (23.6) 112 (4.2)	1869 (71.9) 611 (23.5) 120 (4.6)	1839 (68.8) 686 (25.6) 149 (5.6)	1814 (68.7) 622 (23.6) 203 (7.7)	1518 (57.6) 552 (20.9) 565 (21.4)	<0.0001

SHBG: Sex hormone binding globulin; IQR: interquartile range; SD: standard deviation; MHT: menopausal hormone therapy; Chi-square tests, one-way analysis of variance, or Wilcoxon rank sum tests were used to compare proportions/frequencies, means, and medians as appropriate.

Table 3.2 Hazards of Incident Ischemic Stroke in the Women's Health Initiative by Quintiles of SHBG Levels

	Model 1*	Model 2**	Model 3†
SHBG	HR (95%CI)	HR (95%CI)	HR (95%CI)
Q1	1.88 (1.47-2.41)	1.69 (1.30-2.20)	1.61 (1.19-2.19)
Q2	1.34 (1.03-1.73)	1.27 (0.98-1.65)	1.24 (0.91-1.68)
Q3	1.44 (1.13-1.85)	1.40 (1.09-1.80)	1.44 (1.08-1.92)
Q4	1.49 (1.16-1.91)	1.46 (1.14-1.87)	1.49 (1.12-1.98)
Q5	Reference	Reference	Reference

Q1: Lowest quintile; Q5: Highest quintile; *Adjusted for age, race/ethnicity, SHBG assay as strata variable, p <0.0001 (test for trend); **Adjusted for Model 1 and body mass index, history of hypertension, alcohol use, and smoking status, p=0.004 (test for trend); †Adjusted for Model 2 and physical activity, age at menopause, parity, use of menopausal hormone therapy at baseline, history of using oral contraceptives, age at menarche, p=0.04 (for trend test). Due to missing data for covariates, sample size for Model 3 is 9688.

Table 3.3 Hazards of Incident Ischemic Stroke Across Quintiles of Sex Hormone Binding Globulin Levels, Adjusted for Potential Mediators

	Model 1 with history of diabetes* (N=13184)	**Model 2 with total estradiol** (n=10725)**	**Model 3 with total estradiol and testosterone† (n=5595)**	**Model 4 with CRP‡ (n=5287)**
SHBG	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Q1	1.63 (1.26-2.12)	1.99 (1.49-2.66)	1.56 (0.90-2.70)	1.52 (1.07-2.17)
Q2	1.25 (0.96-1.62)	1.61 (1.20-2.15)	2.15 (1.31-3.51)	1.32 (0.93-1.89)
Q3	1.40 (1.09-1.80)	1.58 (1.19-2.11)	1.65 (1.00-2.73)	1.48 (1.05-2.09)
Q4	1.46 (1.14-1.88)	1.63 (1.23-2.16)	2.06 (1.29-3.31)	1.40 (1.00-1.98)
Q5	Ref	Ref	Ref	Ref

Q1: Lowest quintile; Q5: Highest quintile; All models are adjusted for age, race/ethnicity, body mass index, history of hypertension, alcohol use, and smoking status, along with potential mediators as noted. *Model 1 also stratified by SHBG assay; p=0.01 (test for trend); ** p<0.0001 (test for trend); †p=0.09 (test for trend); ‡p=0.07 (test for trend)

Figure 3.1 Study Participants

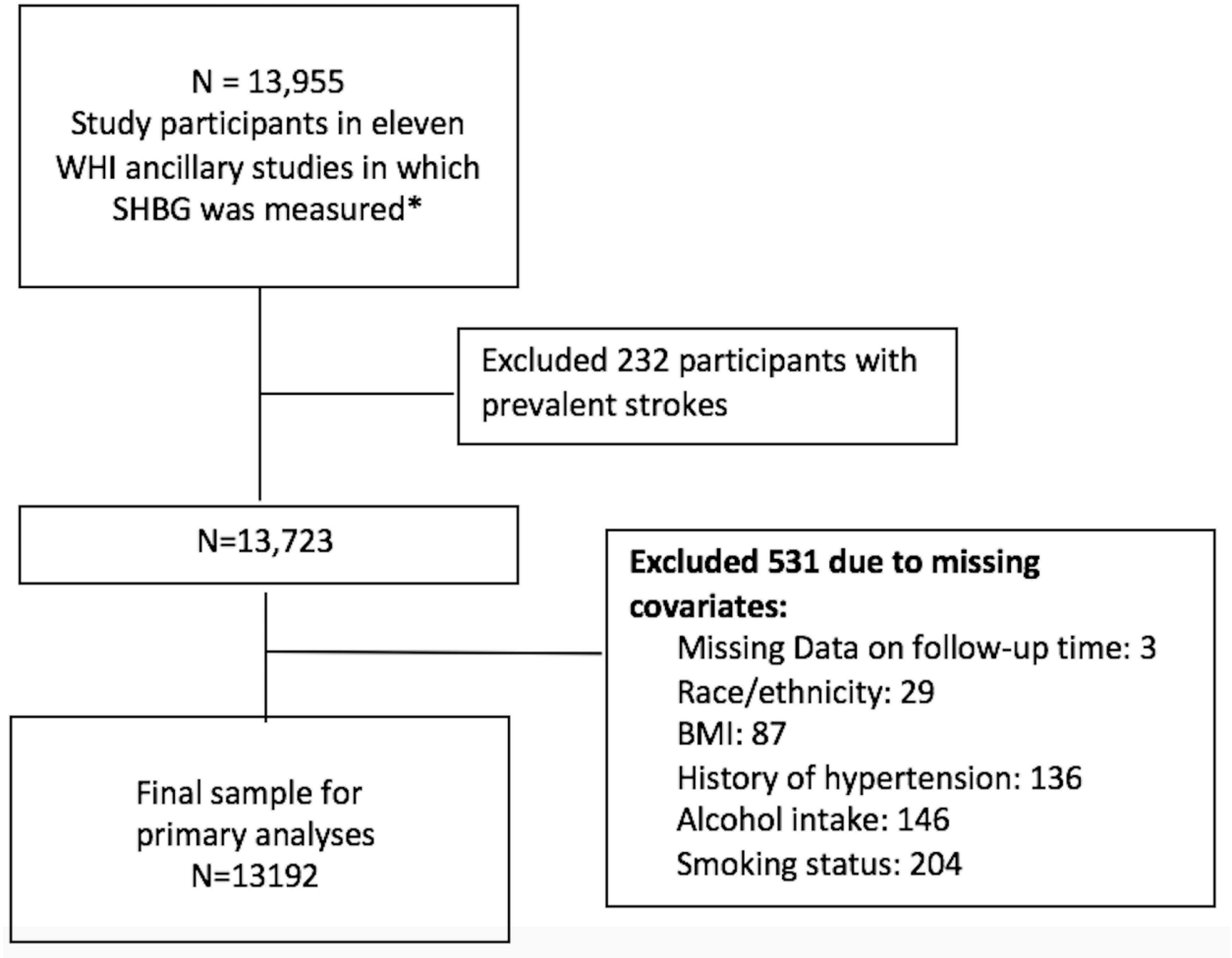


Figure 3.2 A Directed Acyclic Graph linking SHBG to Stroke Risk

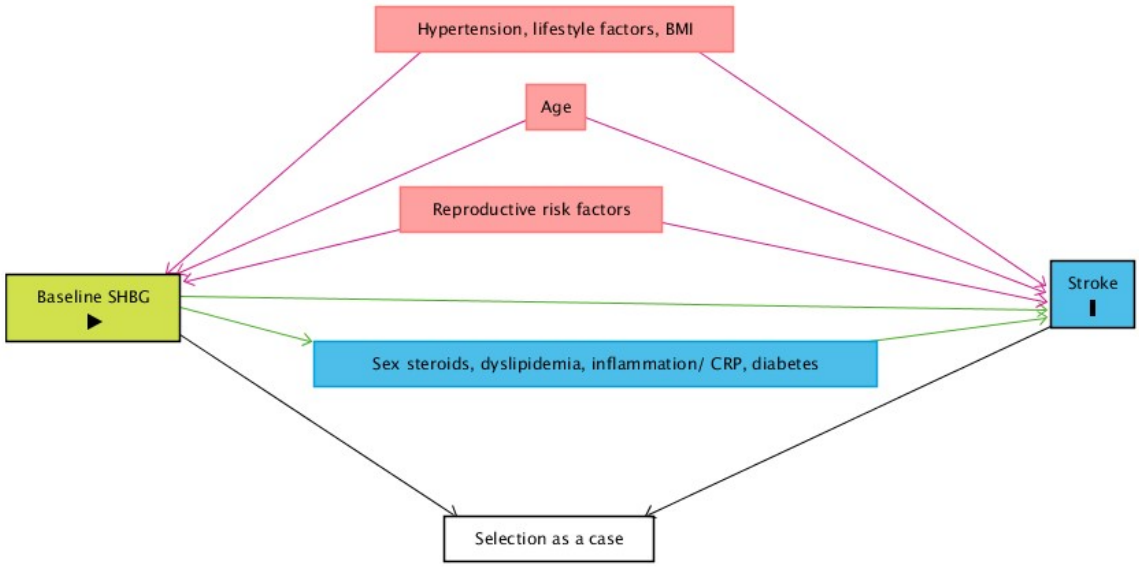
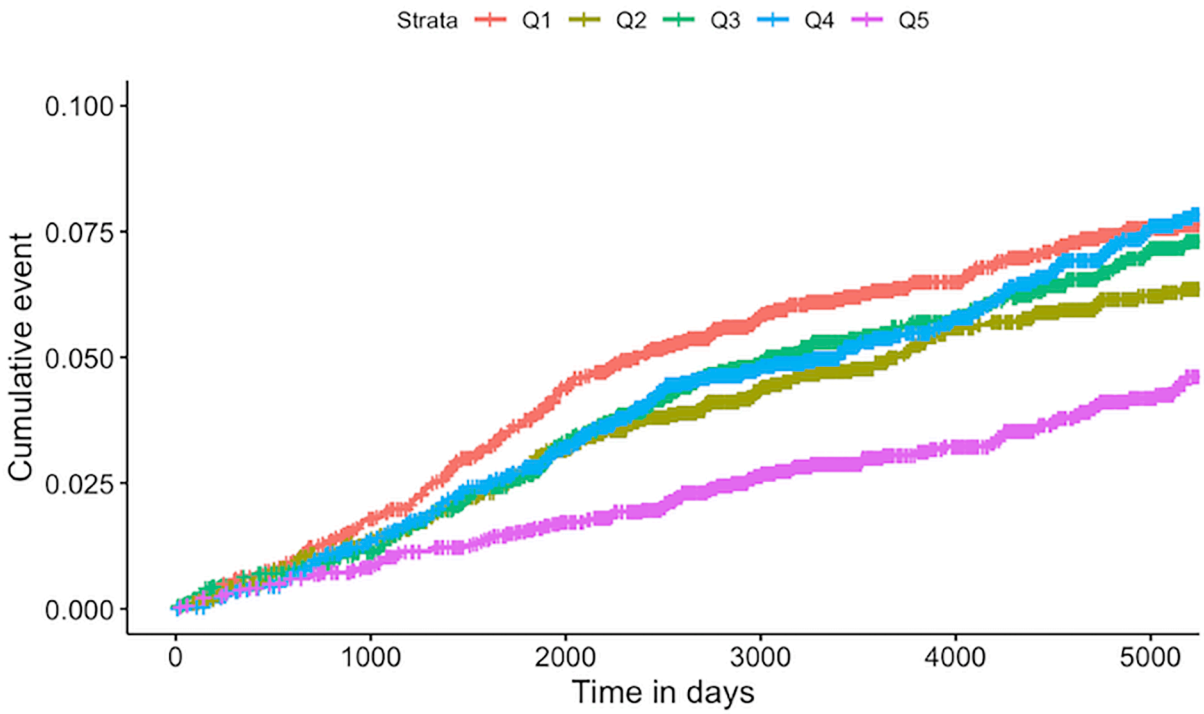
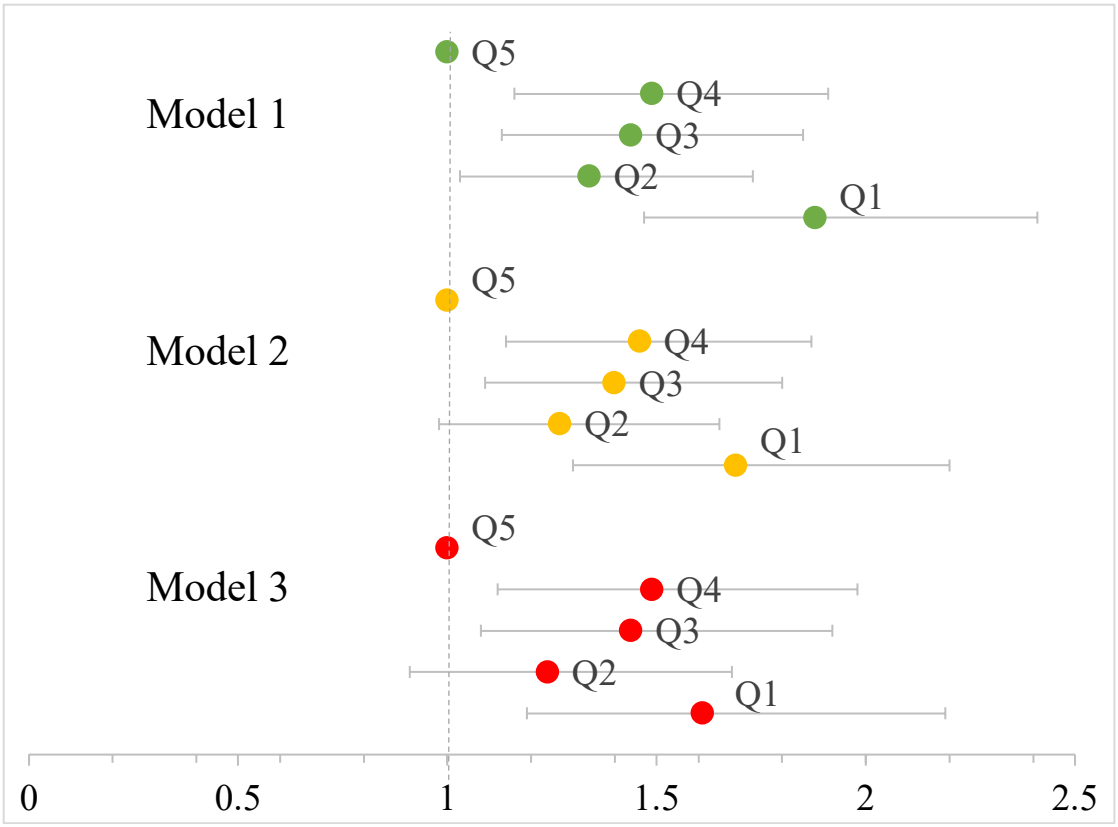


Figure 3.3: Cumulative Hazards of Incident Ischemic Stroke, Unadjusted, by SHBG Quintile



Q1: Lowest SHBG quartile, Q5: Highest SHBG Quartile; Log-rank test $p<0.0001$

Figure 3.4 Hazard Ratios (95% CI) of Incident Ischemic Stroke in the Women's Health Initiative by Sex Hormone Binding Globulin Quintile



Q1 is the lowest SHBG quintile; Q5 is the highest SHBG quintile. Model 1 is adjusted for age and race/ethnicity, Model 2 adjusted for Model 1 plus body mass index, history of hypertension, alcohol use, and smoking status. Model 3 adjusted for Model 2 plus physical activity, age at menopause, parity, use of menopausal hormone therapy at baseline, history of using oral contraceptives, age at menarche. All models are stratified by SHBG measurement assay.

CHAPTER 4: Sex-Specific Stroke Risk Stratification and Prediction

Abstract: Though several risk prediction scores exist, little is known about the potential for improved clinical risk determination for stroke incidence particularly in women using hormonal biomarkers. Our objective was to evaluate the ability of SHBG and reproductive stroke risk factors to predict incident IS in postmenopausal women. The study sample consisted of a sub-cohort of women from the national WHI source population . Women who participated in one of eleven ancillary studies in which baseline serum SHBG was measured were included in this analysis (n=13,723). The outcome was incident IS, as defined in Chapter 3. A sequential, manual selection process was employed, first using variables from the revised Framingham Stroke Risk Score (R-FSRS), followed by the addition of serum SHBG level (log-transformed) and sex-specific reproductive risk factors. Interaction terms were also tested. Cox proportional hazards models were used with the inclusion of candidate risk factors, and performance of each model was tested using discrimination statistics (area under the curve at 5-year intervals and Harrell's C-statistic for survival data). Calibration of each model was tested by comparing observed to predicted stroke risks at 5-year intervals. In our study sample, adding serum SHBG did not improve model performance as measured by AUC or concordance, though overall model discrimination was good (range of c-statistics was 0.68-0.69). Despite this, low serum SHBG was associated with an increase in predicted stroke risk by an average of 36% at 10 years based on survival curves, even after adjustment for model covariates. In conclusion, despite the lack of improved model performance with the addition of SHBG or reproductive risk factors, more work is needed to understand to how mitigate the increased risk associated with low serum SHBG.

Introduction

Several risk scores have been developed for the purposes of predicting risk of ischemic stroke.[47,73–78] These include the Framingham Stroke Risk Score and the Atherosclerotic Cardiovascular Disease (ASCVD). The score most widely used currently is the ASCVD score and is based on the Pooled Cohort Equations from the 2013 AHA/ ASA guidelines. It predicts the 10-year risk of cardiovascular disease (myocardial infarction, coronary heart disease), fatal and non-fatal stroke) broadly and includes age, sex, race, total cholesterol, high-density lipoprotein, systolic blood pressure (SBP), blood pressure treatment, diabetes, and smoking status.[78,79] Though one major improvement of this score over the previous Framingham score is its validity in non-white populations, there have been some weaknesses including poor calibration and overestimation of CVD events.[80–82]

There is a critical need to use strengths of the ASCVD score and previous Framingham scores along with risk factors specific to women, the ability to weight risk factors differently for women vs. men (i.e. hypertension, diabetes), and the possible inclusion of hormone-related biomarkers (i.e. SHBG) in the prediction of stroke.[12] Previous data indicate some ability to increase discriminative ability of risk scores for coronary heart disease by including reproductive risk factors.[83] Though recent iterations of the Framingham Stroke Risk Score, specifically the recalibrated Revised Framingham Stroke Risk Score (R-FSRS), provide sex-specific weights for risk factors as well as sex-specific baseline rates,[84] evidence on sex-specific reproductive risk factors as well as lack of knowledge of the biologic mechanisms for elevated stroke risk in post-menopausal women leaves room for improved risk prediction in this demographic across diverse populations.

Given the previous finding of an inverse association between SHBG and IS described in Chapter 3, the primary objective of this analysis was to evaluate the potential predictive ability of SHBG for incident IS, and an additional objective was to evaluate the potential predictive ability of sex-specific risk factors related to reproductive history for IS when added to traditional risk factors.

Methods

Study Sample

The initial study sample for this chapter was the same as that for Chapter 3, women in a sub-cohort of the larger WHI study who were enrolled into one of eleven ancillary studies in which serum SHBG was measured. For each candidate model described below, sample size varies to some degree due to variability in the number of missing observations for certain covariates.

Outcome and Covariates

As in Chapter 3, the outcome was incident IS as defined as the rapid onset of a persistent neurologic deficit lasting at least 24 hours due to cerebrovascular obstruction. To be included as an event, the relevant deficit must have lasted at least 24 hours or have a compatible lesion on CT and/or MRI. Additional ascertainment and adjudication procedures are described in Chapter 3 as well. Again, as above, participants that did not experience an IS during the observed follow-up period were censored in cases of death, loss to follow-up, or administrative censoring at the end of 2018. Data on model covariates were obtained by self-report at the time of study enrollment with the exception of SBP. SBP was the average of two blood pressure measurements taken during the initial study visit. A detailed description of SHBG measurement can be found in Chapter 3 (pages 41-42).

Descriptive Statistics and Baseline Stroke Risk

Baseline demographics and characteristics describing the study sample were computed using frequencies and proportions for categorical variables, means and standard deviations (SD) for normally distributed variables, and medians with interquartile ranges (IQR) for non-normally distributed variables. Observed probabilities of remaining stroke-free at 5-year intervals and over the entire follow-up period were obtained from Kaplan-Meier product limit estimates and were reported for the overall sample and by SHBG quintile.

Potential Covariates for Risk Scores and Model Selection Process

With regard to traditional risk factors, we utilized factors included in the R-FSRS.[84] These variables include age, SBP, use of current anti-hypertensive medications, an interaction term between SBP and use of current anti-hypertensive medications, whether the participant is a current smoker, prevalent atrial fibrillation, and prevalent CVD. Additional factors from the FSRS including an interaction term between age ≥ 65 years and prevalent diabetes were not included given the age distribution of our sample; the median age in our sample was 66 (IQR 60.0-71.0)). Additional factors that are part of the ASCVD/ pooled cohort equations including total cholesterol and HDL cholesterol were not included due to the unavailability of these data for the majority of participants in the sample. Race was not included as a potential candidate as our objective was to evaluate biologic predictors of stroke rather than social determinants of health that comprise the social constructs of race or ethnicity.[85]

SHBG was log transformed given its non-normal, right-skewed distribution. Candidate factors related to reproductive history included age at menopause in 5-year intervals, age at menarche, parity defined as number of full term pregnancies (defined in the WHI as pregnancies progressing past 6 months of gestational age), age at first pregnancy, history of menstrual

irregularity, having breastfed at least one child, past use of oral contraceptives, past or current use of menopausal hormone therapy (MHT) at baseline, and history of migraine and were based on prior data demonstrating associations with IS or CVD.[86–90]

To achieve our two stated objectives, five models were created: 1) Model 1 included variables from the R-FSRS; 2) Model 2 included R-FSRS variables with the addition of log SHBG as a continuous variable; 3) Model 3 included all variables in Model 1 with the addition of reproductive risk factors with evidence of significant associations with IS ($p<0.10$) in unadjusted Cox models; 4) Model 4 included all variables in Model 3 with the addition of log SHBG in order to further evaluate our primary objective; and 5) Model 5 was designed to be the most parsimonious sex-specific model with reproductive variables with $p>0.10$ removed (based on p-values from the adjusted Model 4). Likelihood ratio tests were used to evaluate the model fit of nested models. Additional terms including body mass index (BMI) as well as multiple interaction terms including age by MHT, age by atrial fibrillation, baseline age by age at menopause, age by log SHBG, and BMI by log SHBG were tested using Wald chi-square tests and likelihood ratio tests.

Prediction Characteristics

Following model selection, we evaluated the discrimination and calibration of each model. To evaluate discrimination, Harrell's concordance statistics (c-statistic) and area under the curve (AUC) were calculated accounting for right-censored data and loss of equivalency of the c-statistic and AUC.[91] Receiver operating curves (ROC) at 5, 10, and 15 years as well as over the full follow-up period were compared as well. For calibration, observed risks of stroke at each time point (5, 10, and 15 years as well as over full follow-up period) were estimated from Kaplan-Meier product limit estimates and were compared to mean predicted probabilities based

on each of the 5 models. Calibration was also assessed across SHBG quintiles. Finally, mean predicted stroke-free survival times were evaluated, stratified by SHBG quintile, to evaluate the calibration across SHBG quintiles.[92]

All analyses were conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC).

Results

Our analytic sample included 13,723 participants followed for a median of 13.7 years, and 968 events over the full follow-up period occurred. Baseline characteristics can be found in Table 4.1. Median age was 66 (IQR 60.0-71.0), and the sample demonstrated diversity by race with 67.0% being White, non-Hispanic and 18.9% categorized as Black. Table 4.2 describes the crude estimated probabilities of remaining stroke-free at 5-year intervals and at the end of the follow-up; at 10 years, 95% of the participants were stroke free, though it should be noted that stroke-free survival at each time point varied by SHBG quintile (Table 4.2), with those in the lowest quintile, or with the lowest SHBG levels, having the lowest probability of being stroke free at 5, 10, and 15 years of follow-up.

Final Models 1 through 5 are displayed in Table 4.3 along with adjusted hazard ratios. Of note, log SHBG demonstrates a significant association with incident IS with an adjusted hazards ratio of 0.84 (95%CI 0.74-0.95) in Model 2 and 0.83 (95% CI 0.72-0.95) in Model 4. The reproductive risk factor related variables that met criteria for model entry were age at menopause, number of full-term pregnancies, history of breastfeeding at least one child for at least one month, history of MHT, and history of oral contraceptive use. However, once adjusted by other traditional risk factors, only age at menopause remained significant to $p<0.10$. Model 5, the most parsimonious model, consisted of the R-FSRS variables, age at menopause, and log SHBG. Of note, atrial fibrillation did not meet statistical significance for association with stroke

but was kept in the model to parallel the Framingham Risk Score and to increase the chance of external validity of the score in other populations.

Figure 4.1 (Panels A through E) demonstrate the discrimination characteristics of each of the 5 models. C-statistics as well as AUC estimates are similar across 5 models and indicate good discriminative ability. C-statistics for Models 1, 2, 3, 4, and 5, were 0.686, 0.686, 0.690, 0.691, and 0.686 respectively.

Figures 4.3 and 4.4 demonstrate the calibration characteristics of each model, observed vs predicted stroke probabilities at 5-year intervals overall and by SHBG quintile and demonstrate adequate calibration overall, with some over estimation of stroke risk in the highest SHBG quintile (5.3-5.5% predicted vs. 3.7% observed) and under estimation of stroke risk in the lowest SHBG quintile (5.0-5.1% predicted vs. 5.9% observed).

The average predicted survival based on Model 5 and stratified by SHBG quintile, are displayed in Figure 4.5 and demonstrate a difference in probability of remaining stroke free between quintiles. For example, At year 10, the probability of remaining stroke free for those in the lowest quintile of SHBG was 1.7% lower than those in the highest quintile of SHBG, representing a 36% increase.

Discussion

In summary, though our sex-specific stroke prediction models, including those with SHBG as a predictor variable, had reasonably good prediction abilities for incident IS, the addition of SHBG did not appear to increase accuracy of prediction. Specifically, despite demonstrating that SHBG had a statistically significant association with acute IS in our prediction models, the discriminative ability did not improve from the risk score with traditional risk factors. One potential explanation may be the relatively small effect size between SHBG and

stroke when compared with factors such as SBP, age, and diabetes. It is also possible that low SHBG could be exerting an effect on IS risk through some of these traditional risk factors like hypertension (i.e., mediation), making the addition of SHBG to traditional risk scores less critical. For example, if low SHBG results in a higher testosterone to estradiol ratio, vasoconstriction and pro-inflammatory processes could lead to increased SBP and increased stroke risk. It must be noted, however, that even when adjusting for the major stroke risk factors in our risk model (i.e., hypertension, age, diabetes, and smoking), there was still a relatively large increase in risk between those in the lowest and highest quintiles of SHBG, about 37% at both 10 and 15-year time points. This equates to about a 2% absolute increased risk, which is not inconsequential given the disability associated with stroke.

Another possible explanation for our findings is that the impact of reproductive risk factors and circulating hormone levels may differ over the life course. For example, reproductive risk factors may be more critical to the prediction of stroke and CVD for pre-menopausal women, before the appearance of co-morbidities such as clinical diabetes or when factors such as hypertension are milder. It is also known that the effect of sex hormones such as estradiol on endovascular function differ over the life course, with the benefits of estrogen including vasodilation and anti-inflammation decreasing with age. Further work on genetic and non-genetic predictors of stroke risk in postmenopausal women independent of circulating sex hormones is warranted.

Limitations and Strengths

Data on other reproductive risk factors that could add to the predictive ability of our risk score – such as pre-eclampsia – were only collected on a small proportion of the WHI participants and thus could not be included in this analysis. In addition, as evident from Chapters

3 and 4, the nature of the relationship between serum SHBG and incident stroke risk varies from the expected dose response pattern to some degree, potentially indicating a non-linear relationship that would be difficult to incorporate into a risk score.

Despite being a sub-cohort of the WHI, compared with other sub-cohorts of postmenopausal women from REGARDS and Framingham, our sample of post-menopausal women showed similar prevalence rates of major stroke risk factors including age, SBP, current smoking, diabetes, and atrial fibrillation,[84] making it likely that our sample of women is representative of postmenopausal women of diverse backgrounds in the United States. Another benefit of the study sample we used is the racial diversity of participants, indicating representativeness with respect to the greater United States population.

Conclusion

In a diverse cohort of women enrolled in the WHI, serum SHBG levels and reproductive risk factors did not materially improve performance of risk models when added to traditional risk factors, despite being statistically significant predictors of incident IS. Though the association between low SHBG and elevated stroke risk in the WHI remains, more work is needed to expand the pool of risk predictors to other biomarkers, genetic or otherwise, that may help improve sex-specific stroke risk prediction.

Tables and Figures

Table 4.1 Baseline Characteristics of Study Participants

Variable	Overall SHBG Sub cohort (n= 13723)
Baseline age, median (IQR)	66.0 (60.0-71.0)
Race, n (%)	
American Indian/ Alaska Native	122 (0.9)
Asian / Pacific Islander	657 (4.8)
Black	2588 (18.9)
Hispanic/Latino	1052 (7.7)
White (non-Hispanic)	9170 (67.0)
Other	105 (0.77)
History of Hypertension, n (%)	5325 (39.2)
BMI, median (IQR)	27.9 (24.5-32.2)
Systolic blood pressure, mean (SD)	130.5 (18.2)
Use of antihypertensives currently	3932 (29.7)
Prevalent CVD	2265 (17.8)
Current smoker, n (%)	1173 (8.7)
History of atrial fibrillation, n(%)	620 (4.6)
History of diabetes	1081 (7.9)
Use of MHT, n (%)	
Never	9317 (67.9)
Past	3209 (23.4)
Current	1191 (8.9)
Age at menopause in years, median (IQR)	50.0 (45.0-52.0)
Number of full-term pregnancies, n (%)	
0	2934 (21.8)
1	1174 (8.7)
2-4	7226 (53.6)
≥5	2157 (16.0)
History of breastfeeding, n (%)	7121 (52.6)
History of oral contraceptive use, n (%)	4661 (34.0)

SHBG: sex hormone binding globulin; IQR: interquartile range; BMI: body mass index; SD: standard deviation; CVD: cardiovascular disease; MHT: menopausal hormone therapy

Table 4.2 Observed Stroke Events and Probability of Remaining Stroke Free

			By SHBG Quintile									
	Overall Sample (n=13723)		Q1		Q2		Q3		Q4		Q5	
	n	(S_t)	n=2713	S_t	n=2773	S_t	N=2740	S_t	N=2754	S_t	N=2742	S_t
5 years	373	0.97	93	0.97	83	0.97	67	0.98	75	0.97	55	0.98
10 years	652	0.95	150	0.94	135	0.95	131	0.95	142	0.94	94	0.96
15 years	814	0.93	180	0.92	164	0.93	162	0.93	187	0.92	121	0.94
Total follow-up	968	0.90	220	0.88	196	0.90	190	0.90	218	0.89	144	0.92

S_t: Observed probability of remaining stroke free from Kaplan Meier estimator; IS: ischemic stroke; Q1, first quintile, represents lowest values of serum SHBG.

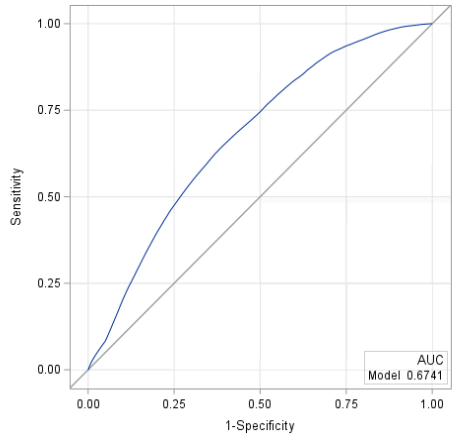
Table 4.3 Sequential Prediction Models for Incident Ischemic Stroke, Adjusted Hazard Ratios with 95% Confidence Intervals

Variables	Model 1	Model 2	Model 3	Model 4	Model 5
Age (per 5 years)	1.32 (1.25-1.39) ††	1.33 (1.27-1.41) ††	1.31 (1.23-1.39) ††	1.32 (1.25-1.40) ††	1.33 (1.26-1.40) ††
SBP (per 10 mm Hg) among those on antihypertensives	1.09 (1.03 1.15) †	1.09 (1.03-1.15) †	1.10 (1.03-1.16) †	1.10 (1.04-1.17) †	1.10 (1.04-1.17) †
SBP (per 10 mm Hg) among those not on antihypertensives	1.22 (1.17-1.29) †	1.22 (1.16-1.28) †	1.23 (1.17-1.29) †	1.22 (1.16-1.29) †	1.22 (1.16-1.28) †
History of diabetes	1.61 (1.29-1.99) ††	1.54 1.24-1.91) ††	1.66 (1.32-2.08) ††	1.59 (1.27-1.99) ††	1.60 (1.27-2.00) ††
Current smoker	1.97 (1.58-2.46) ††	2.01 (1.61-2.51) ††	2.02 (1.60-2.54) ††	2.10 (1.64-2.60) ††	2.08 (1.65-2.61) ††
History of CVD	1.41 (1.18-1.68) ††	1.40 (1.17-1.68) ††	1.30 (1.10-1.55) †	1.29 (1.07-1.57) †	1.31 (1.08-1.59) †
History of Atrial Fibrillation	0.92 (0.67-1.27)	0.92 (0.67-1.26)	1.02 (0.74-1.43)	1.02 (0.73-1.42)	1.02 (0.73-1.42)
Age at menopause	N/A	N/A	0.95 (0.90-1.00)*	0.95 (0.90-1.01)*	0.96 (0.91-1.01)*
Number of full-term pregnancies					
0 (Ref)	N/A	N/A	1.0	1.0	N/A
1			1.14 (0.85-1.53)	1.15 (0.86-1.54)	
2-4			1.13 (0.93-1.38)	1.13 (0.93-1.38)	
≥5			1.34 (1.05-1.70)	1.32 (1.04-1.68)	
History of breastfeeding ≥ 1 month	N/A	N/A	1.12 (0.96-1.30)	1.12 (0.96-1.30)	N/A
History of MHT					
Current	N/A	N/A	1.0 (ref)	1.0	N/A
Past			1.22 (0.89-1.65)	1.10 (0.8-1.51)	
Never			1.15 (0.86-1.53)	1.04 (0.78-1.39)	
History of oral contraceptive use	N/A	N/A	1.0 (0.86-1.20)	1.01 (0.85-1.19)	N/A
Log SHBG	N/A	0.84 (0.74-0.95) †	N/A	0.83 (0.72-0.95) †	0.82 (0.72-0.94) †

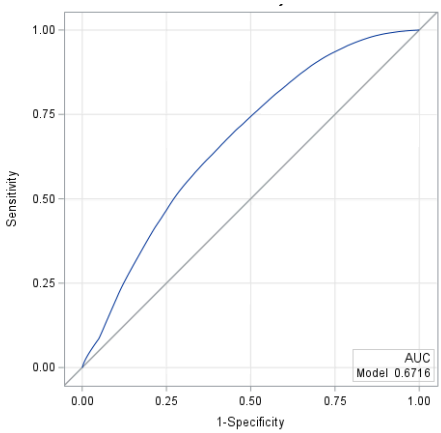
SBP: systolic blood pressure, CVD: cardiovascular disease, SHBG: sex hormone binding globulin. Empty cells represent variables that were not included in specific models. Model 1: n=12375, product term for SBP by anti-hypertensives: 0.0013, Model 2: n=12374, product term = 0.0021; Model 3: n=11250, product term 0.005; Model 4: n=11249, product term p=0.008, Model 5: n=11249, 0.008; P-values: *p<0.10,** p<0.05, †p<0.01, ††p<0.001

Figure 4.1 Receiving Operator Curves and Area Under the Curve at 10 years for Sequential Models

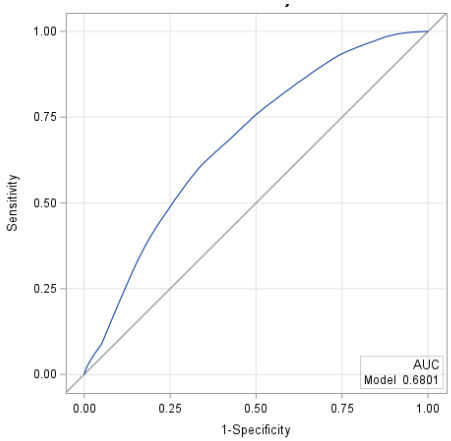
Panel A: Model 1



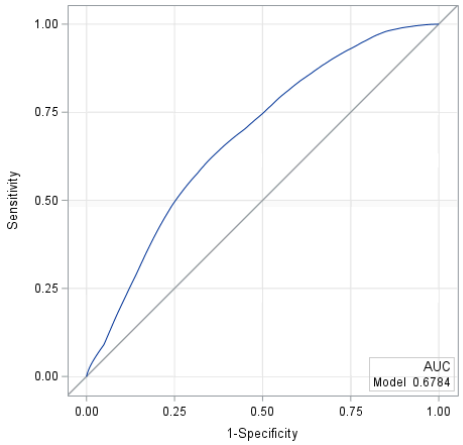
Panel B: Model 2



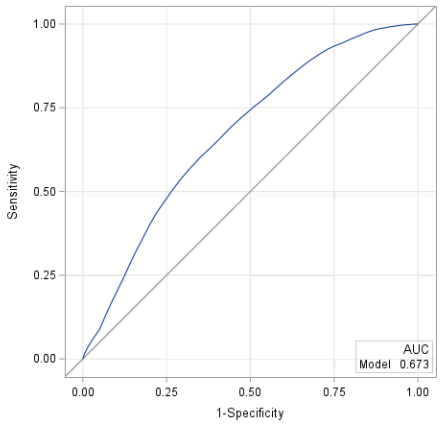
Panel C: Model 3



Panel D: Model 4

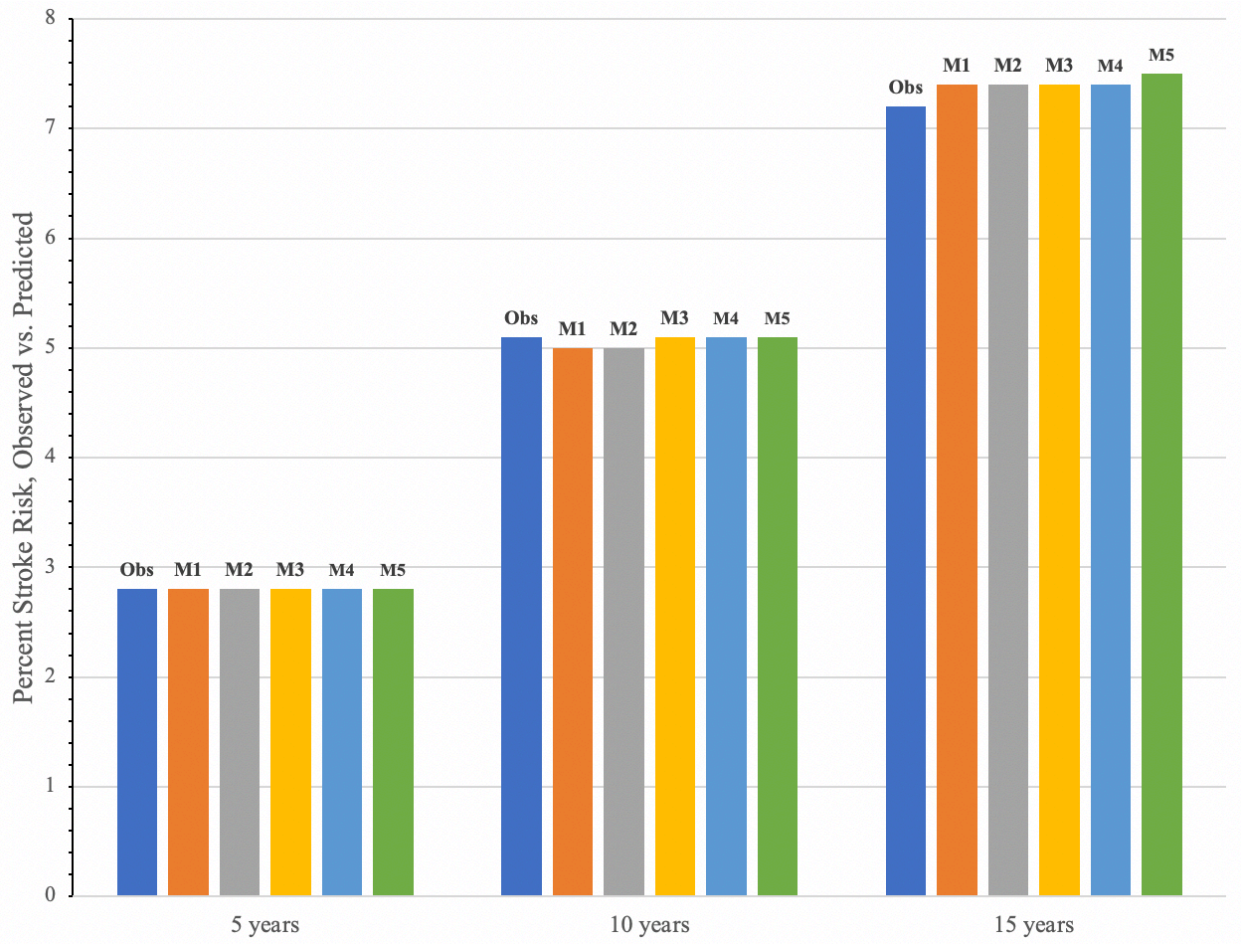


Panel E: Model 5



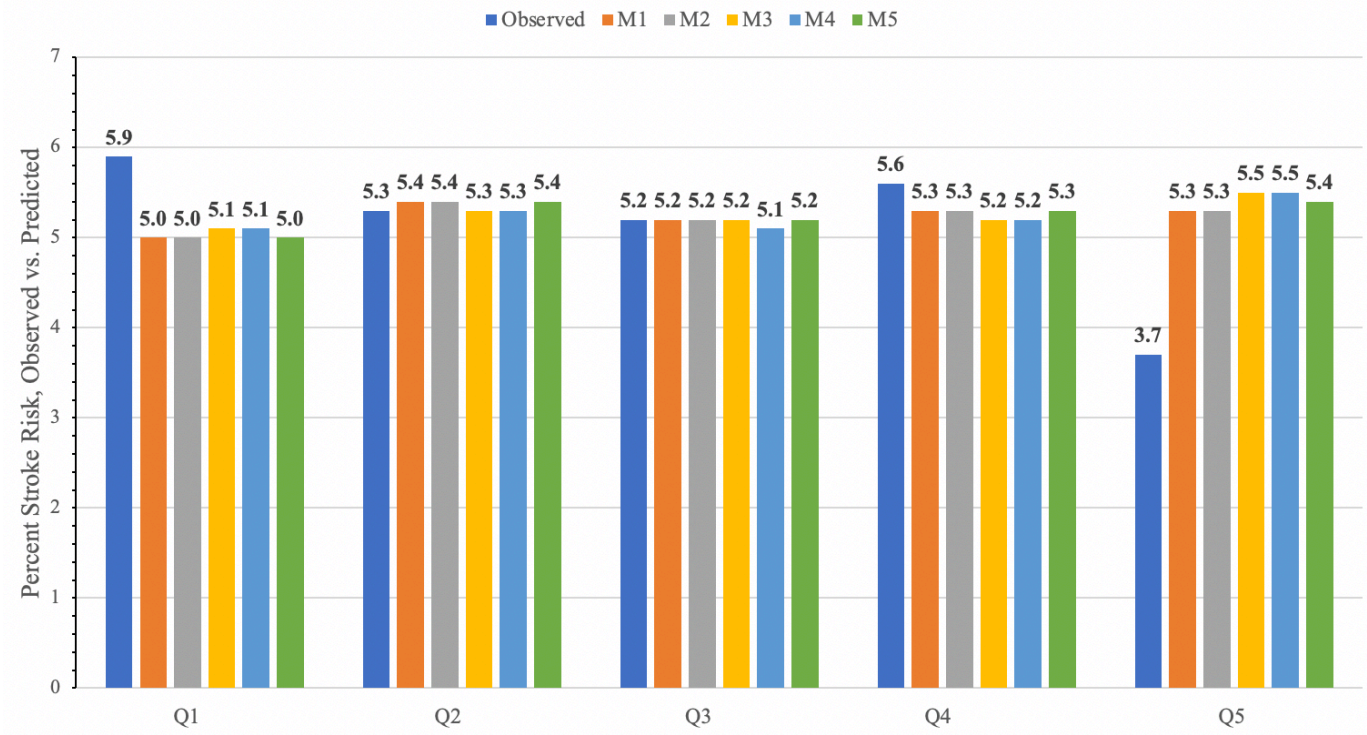
Model 1 includes variables from the revised Framingham stroke risk score, c-statistic: 0.686; Model 2 includes variables from Model 1 plus serum SHBG, c-statistic: 0.686; Model 3 includes Model 1 variables plus reproductive risk factors, c-statistic: 0.690; Model 4 includes Model 3 variables plus serum SHBG, c-statistic: 0.691; Model 5 includes Model 2 variables plus age at menopause; C-statistic = .686

Figure 4.2 Risk of Ischemic Stroke, Observed vs. Predicted Across Models and Time Periods



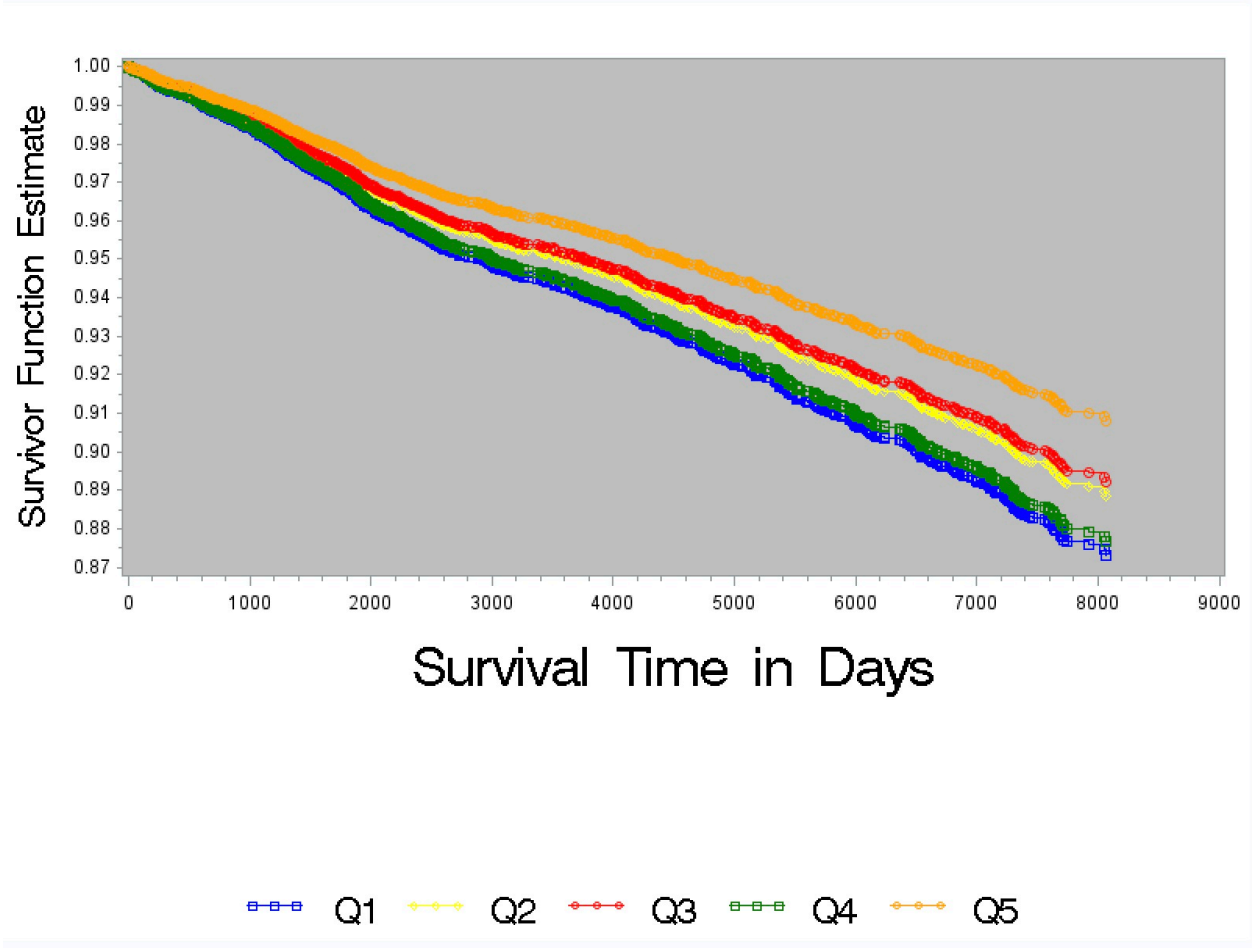
'Obs' refers to observed stroke risk from Kaplan Meier estimator, M1 through M5 refers to predicted risks from Models 1 through 5. Percent stroke risk is calculated as (1 - probability of remaining stroke free)*100

Figure 4.3: Risk of Ischemic Stroke, Observed vs. Predicted at 10 years Across SHBG Quintiles



Q1: First quintile of serum SHBG, Q2: Second quintile of SHBG, Q3: Third quintile of SHBG, Q4: Fourth quintile of SHBG, Q5: Fifth quintile of SHBG. For each quintile, Observed refers to observed stroke risk from Kaplan Meier estimator, M1 through M5 refers to predicted risks from Models 1 through 5.

Figure 4.4 Average Predicted Probability of Remaining Stroke Free Over Time, by SHBG Quintile



Q1: First quintile of serum SHBG, Q2: Second quintile of SHBG, Q3: Third quintile of SHBG, Q4: Fourth quintile of SHBG, Q5: Fifth quintile of SHBG.

THESIS CONCLUSIONS

The above findings demonstrate notable sex differences in stroke risk associated with modifiable risk factors, particularly hypertension and impaired glucose tolerance. Chapter 3 and 4 demonstrate an elevated risk of ischemic stroke associated with low SHBG, a sex hormone and steroid transporter that seems to serve as a marker of cardiometabolic health. Questions remain, however, about this association in populations other than the WHI, how to best use this inverse association to understand the biology of stroke in post-menopausal women, and how to improve prediction tools. A more accurate understanding of the impact of sex and gender on stroke risk and outcomes is needed to move toward more personalized stroke prevention and care across the life course. Specifically, more data in humans elucidating the contributions of both circulating hormones and genetic differences between women and men is needed. Such data could help to address issues of potential confounding and/or selection biases, could help resolve remaining questions about the effects of circulating hormones on stroke risk, could identify novel therapeutic targets for prevention, and could lay the groundwork for sex-specific approaches to stroke care.

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APPENDIX: SUPPLEMENTAL TABLES

Chapter 1, Table A1, Additional Framingham Stroke Risk Factors by Sex and Systolic Blood Pressure Category

	Women (n=13,566)				Men (n=11,117)			
	Systolic Blood Pressure				Systolic Blood Pressure			
Risk Factor	**<120 (n = 4,735) N (%)**	**120-129 (n = 3,664) N (%)**	**130-139 (n = 2,631) N (%)**	**>140 (n = 2,536) N (%)**	**<120 (n = 3,140) N (%)**	**120-129 (n = 3,084) N (%)**	**130-139 (n = 2,488) N (%)**	**>140 (n = 2,405) N (%)**
Diabetes	609 (13)	742 (20)	641 (24)	713 (28)	561 (18)	551 (18)	610 (25)	719 (30)
Current smoking	727 (15)	452 (12)	364 (14)	375 (15)	415 (13)	378 (12)	335 (13)	387 (16)
Atrial Fibrillation	359 (8)	272 (7)	212 (8)	238 (9)	289 (9)	252 (8)	192 (8)	230 (10)
Prevalent cardiovascular disease	432 (9)	415 (11)	355 (13)	407 (16)	685 (22)	657 (21)	587 (24)	623 (26)

Diabetes was defined as fasting glucose ≥ 126 ml/l (or ≥ 200 ml/dl non-fasting) or being on current medications for diabetes. Atrial fibrillation was defined as either ECG evidence of atrial fibrillation or if participants reported being diagnosed by a physician. Prevalent cardiovascular disease was defined as self-reported MI, CABG, bypass, angioplasty, or stenting OR evidence of MI via ECG.

Chapter 1, Table A2, Sex-specific Associations between Increasing Systolic Blood Pressure, Number of Classes of Blood Pressure Medications, and Risk of Ischemic Stroke, Adjusted for Framingham Stroke Risk Factors

Systolic Blood Pressure		No Medications	1 class of medications	2 classes of medications	3 classes of medications	Change per class	Pooled effect of medications, and p-value for effect modification by sex
Normal	W	1.00 (ref)	1.23 (0.72 – 2.09)	1.96 (1.21 – 3.18)	2.34 (1.40 – 3.92)	1.35 (1.15 – 1.59)	
	M	1.00 (ref)	0.99 (0.58 – 1.71)	1.25 (0.74 – 2.10)	2.01 (1.20 – 3.36)	1.25 (1.05 – 1.48) P = 0.49	W: 1.14 (1.04 – 1.24) M: 1.12 (1.03 – 1.21) P = 0.76
Elevated	W	1.25 (0.77 – 2.05)	1.60 (0.99 – 2.60)	1.62 (1.01 – 2.62)	1.17 (0.62 – 2.20)	1.02 (0.85 – 1.22)	
	M	1.17 (0.74 – 1.85)	1.93 (1.24 – 3.01)	1.43 (0.88 – 2.32)	1.36 (0.78 – 2.35)	1.04 (0.89 – 1.21) P = 0.87	
Stage 1	W	1.92 (1.16 – 3.17)	2.28 (1.43 – 3.65)	1.88 (1.17 – 3.04)	2.70 (1.65 – 4.40)	1.08 (0.91 – 1.28)	
	M	1.32 (0.82 – 2.14)	1.44 (0.89 – 2.35)	1.86 (1.17 – 2.95)	2.15 (1.33 – 3.48)	1.19 (1.02 – 1.39) P = 0.42	
Stage 2	W	2.72 (1.66 – 4.46)	2.38 (1.52 – 3.72)	2.53 (1.61 – 3.95)	3.49 (2.24 – 5.43)	1.11 (0.95 – 1.29)	
	M	2.01 (1.26 – 3.20)	1.35 (0.83 – 2.21)	1.86 (1.18 – 2.94)	2.08 (1.31 – 3.01)	1.05 (0.90 – 1.22) P = 0.62	
Change per class	W	1.41 (1.20 – 1.65)	1.25 (1.07 – 1.46)	1.11 (0.95 – 1.29)	1.24 (1.05 – 1.47)		
P-value for effect modification by sex	M	1.25 (1.07 – 1.45) P = 0.29	1.04 (0.89 – 1.22) P = 0.10	1.15 (0.99 – 1.35) P = 0.73	1.06 (0.90 – 1.25) P = 0.19		
Pooled effect of BP	W	1.24 (1.15 – 1.35)					
	M	1.13 (1.04 – 1.23)					
		P = 0.084					

W: Women; M: men; BP: blood pressure

Chapter 1, Table A3: Sex-specific Associations (Hazard Ratios) between Increasing Systolic Blood Pressure, Number of Classes of Blood Pressure Medications, and Risk of Ischemic Stroke Among Black Participants

Systolic Blood Pressure		No Medications	1 class of medications	2 class of medications	3 class of medications	Change per class	Pooled effect of medications*
Normal	W	1.00 (ref)	1.93 (0.83 – 4.47)	1.36 (0.55 – 3.35)	3.24 (1.43 – 7.30)	1.38 (1.07 – 1.80)	W: 1.20 (1.07 – 1.34) M: 1.24 (1.13 – 1.37) P = 0.63
	M	1.00 (ref)	0.82 (0.29 – 2.39)	0.84 (0.31 – 2.24)	0.77 (0.25 – 2.39)	0.92 (0.65 – 1.29) P = 0.060	
Elevated	W	0.83 (0.30 – 2.28)	2.09 (0.95 – 4.61)	2.93 (1.41 – 6.12)	1.86 (0.77 – 4.48)	1.27 (0.99 – 1.63)	
	M	0.73 (0.31 – 1.73)	1.02 (0.43 – 2.41)	1.47 (0.67 – 3.22)	1.18 (0.48 – 2.89)	1.21 (0.91 – 1.60) P = 0.80	
Stage 1	W	1.88 (0.78 – 4.52)	2.59 (1.18 – 5.66)	2.43 (1.13 – 5.20)	4.08 (1.93 – 8.64)	1.26 (1.00 – 1.59)	
	M	0.89 (0.38 – 2.12)	1.23 (0.55 – 2.73)	1.69 (0.81 – 3.55)	1.61 (0.73 – 3.53)	1.22 (0.95 – 1.58) P = 0.87	
Stage 2	W	2.70 (1.14 – 6.36)	3.52 (1.70 – 7.29)	3.21 (1.55 – 6.62)	4.73 (2.32 – 9.65)	1.17 (0.96 – 1.43)	
	M	1.54 (0.70 – 3.38)	0.94 (0.40 – 2.17)	1.52 (0.72 – 3.21)	2.15 (1.07 – 4.34)	1.20 (0.95 – 1.51) 0.91	
Change per class	W	1.46 (1.09 – 1.94)	1.24 (1.00 – 1.53)	1.21 (0.99 – 1.48)	1.24 (1.01 – 1.52)		
	M	1.17 (0.89 – 1.54) P = 0.28	1.04 (0.76 – 1.41) P = 0.35	1.16 (0.90 – 1.49) P = 0.80	1.38 (1.04 – 1.84) P = 0.54		
Pooled effect of BP†	W	1.26 (1.13 – 1.41)					
	M	1.19 (1.03 – 1.36) P = 0.51					

W: Women; M: men; BP: blood pressure

*Across strata of blood pressure, 3 degree freedom test for differences within sex, p=0.81 (women), p=0.54 (men)

† Across strata of medications, p= 0.76 (women), P=0.59 (men)

Chapter 1, Table A4: Sex-specific Associations between Increasing Systolic Blood Pressure, Number of Classes of Blood Pressure Medications, and Risk of Ischemic Stroke Among White Participants

Systolic Blood Pressure		No Medications	1 class of medications	2 class of medications	3 class of medications	Change per class	Pooled effect of medications*
Normal	W	1.00 (ref)	0.98 (0.50 – 1.92)	2.92 (1.72 – 4.97)	2.60 (1.33 – 5.07)	1.49 (1.23 – 1.81)	
	M	1.00 (ref)	1.33 (0.72 – 2.47)	1.92 (1.06 – 3.50)	3.98 (2.24 – 7.07)	1.56 (1.29 – 1.89) P = 0.74	W: 1.26 (1.13 – 1.41)
Elevated	W	1.24 (0.72 – 2.14)	1.63 (0.91 – 2.91)	1.14 (0.57 – 2.27)	1.15 (0.48 – 2.76)	0.97 (0.76 – 1.24)	M: 1.19 (1.03 – 1.36)
	M	1.40 (0.83 – 2.37)	2.86 (1.72 – 4.76)	1.95 (1.90 – 3.49)	1.99 (1.02 – 3.91)	1.11 (0.93 – 1.33) P = 0.37	P = 0.51
Stage 1	W	1.84 (1.02 – 3.34)	2.69 (1.54 – 4.70)	1.86 (0.99 – 3.51)	3.25 (1.70 – 6.23)	1.13 (0.90 – 1.42)	
	M	1.72 (0.98 – 3.01)	1.86 (1.03 – 3.36)	2.53 (1.45 – 4.42)	3.78 (2.14 – 6.67)	1.30 (1.08 – 1.57) P = 0.34	
Stage 2	W	2.57 (1.42 – 4.65)	2.22 (1.25 – 3.94)	2.73 (1.50 – 4.95)	3.94 (2.19 – 7.10)	1.17 (0.84 – 1.46)	
	M	2.27 (1.28 – 4.00)	2.12 (1.19 – 3.76)	2.69 (1.54 – 4.70)	2.64 (1.41 – 4.96)	1.08 (0.88 – 1.31) P = 0.58	
Change per class	W	1.37 (1.14 – 1.66)	1.31 (1.07 – 1.61)	0.99 (0.79 – 1.24)	1.27 (0.98 – 1.63)		
	M	1.30 (1.09 – 1.56) P = 0.69	1.08 (0.90 – 1.29) P = 0.15	1.14 (0.84 – 1.37) P = 0.34	0.94 (0.76 – 1.15) P = 0.072		
Pooled effect of BP†	W	1.24 (1.11 – 1.38)					
	M	1.12 (1.02 – 1.23)					
		P = 0.17					

W: Women; M: men; BP: blood pressure

*Across strata of BP, 3 degree freedom test for differences within sex, p=0.045 (women), p=0.026 (men)

†Across strata of medications, p= 0.14 (women), P=0.12 (men)

Chapter 2, Table A5: Estimates for Risk of Ischemic Stroke Associated with Increasing Fasting Blood Glucose by Race/ Sex Group among Women with Data on Use of Menopausal Hormone Therapy

Fasting Blood Glucose	**Model 4: Women with data on use of menopausal hormone therapy (N=11224) Adjusted Hazard Ratios (95% CI)**	**Model 5: Only Women who report never taking menopausal hormone therapy (N=4861) Adjusted Hazard Ratios (95% CI)**
<100 (Referent)		
BW	1.0†	1.0
WW	1.0†	1.0†
Overall HR	1.0†	1.0†
100-125 mg/dL		
BW	1.16 (0.84, 1.60)	1.03 (0.65, 1.63)
WW	1.40 (1.04, 1.88)	1.50 (0.91, 2.47)
OVERALL HAZARD RATIO	1.29 (1.03, 1.62)	1.23 (0.87, 1.74)
126-149 mg/dL		
BW	1.13 (0.65, 1.95)	1.50 (0.76, 2.97)
WW	2.69 (1.70, 4.27)	4.00 (2.13, 7.52)
OVERALL HAZARD RATIO	1.78 (1.22, 2.58)	2.40 (1.47, 3.93)
≥150 mg/dL		
BW	1.54 (0.96, 2.48)	1.36 (0.71, 2.63)
WW	1.84 (1.06, 3.17)	2.36 (1.11, 5.02)
OVERALL HAZARD RATIO	1.71 (1.16, 2.52)	1.75 (1.02, 3.02)
P-value for race/sex by FBG interaction term	0.092*	0.1274

BW: Black women, WW: white women
Model 4 adjusted for age, age*race/sex, race/sex, medication use (no diabetes medication, oral diabetes medication, insulin use), BMI, systolic blood pressure, use of anti-hypertensive medications, left ventricular hypertrophy, atrial fibrillation, history of coronary artery disease, smoking, education, income, and ever use menopausal hormone therapy
Model 5: Restricted to only women who have never taken hormone therapy, includes all other covariates as in Model 4.
*Prespecified $P < 0.10$ considered statistically significant for interaction terms; †P-value <0.05 for linear trend

Chapter 3, Table A6: Participants in the Women's Health Initiative (WHI) Ancillary Studies with Measures of Circulating SHBG Levels

Ancillary Study Number	**Description of Cases/ Controls**
90	400 hip fracture, 400 controls
110	385 coronary heart disease (CHD), 385 controls
167	311 breast cancer, 592 controls
238*	700 type II diabetes, 1400 controls
W9	750 controls, 750 hip fractures
BA7	753 coronary heart disease, 534 stroke, 422 venous thromboembolism, 204 spine fracture, 830 fracture excluding spine or hip, 873 controls
BA9	1132 general fracture, 1132 control
BA21	400 colorectal cancer, 800 controls
W5**	150 DM intervention, 150 DM controls at B and Y1
W10	755 breast cancer, 755 controls
W18	120 HT hormone pretest active controls, 120 placebo controls

*Conducted using electrochemiluminescence immunoassays from Roche Diagnostics at UCLA.
** IRMA-immunoradiometric assay using monoclonal antibody labeled with (125) at Esoterix Laboratory Services Inc. (Calabasas Hills, CA).
All other ancillary studies were conducted using Chemiluminescent Immunoassay/radioimmunoassay from Siemens Medical Solutions Diagnostics at the Reproductive Endocrine Research Laboratory at the University of Southern California.

Chapter 3, Table A7: Estimates for Hazards of Incident Ischemic Stroke in the WHI by SHBG Quintiles, Adjusted for a History of Liver Disease

	Model 1*	Model 2**	Model 3†
SHBG	HR (95%CI)	HR (95%CI)	HR (95%CI)
Q1	1.88 (1.47-2.41)	1.69 (1.31-2.19)	1.61 (1.19-2.19)
Q2	1.34 (1.03-1.73)	1.27 (0.98-1.65)	1.24 (0.91-1.68)
Q3	1.45 (1.13-1.85)	1.40 (1.09-1.80)	1.44 (1.08-1.92)
Q4	1.49 (1.16-1.91)	1.46 (1.14-1.87)	1.49 (1.12-1.98)
Q5	Reference	Reference	Reference

Q1: Lowest quintile; Q5: Highest quintile; *Adjusted for age, race/ethnicity, history of liver disease, SHBG assay as strata variable; **Adjusted for Model 1 and body mass index, history of hypertension, alcohol use, and smoking status; †Adjusted for Model 2 and physical activity, age at menopause, parity, use of menopausal hormone therapy at baseline, history of using oral contraceptives, age at menarche.