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Stroke and the Female Brain

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Summary and Introduction

Summary

Stroke is a major public health problem. The female population carries a higher stroke burden than the male population, both because females have a longer life expectancy and because most stroke deaths occur in women. Differences between the sexes in relation to stroke are increasingly being recognized; for example, among stroke survivors, women tend to have worse outcomes than men, as indicated by more-severe disability and an increased likelihood of institutionalization in women. Women and men with stroke also differ in their risk factor profiles, and they respond differently to primary-prevention and acute stroke treatment. Women experience variations in endogenous estrogens throughout their life cycle and might also be exposed to exogenous estrogens, both of which markedly affect the brain. An understanding of the effects of endogenous and exogenous estrogens on cerebral hemodynamics could guide research into explaining how hormone therapy increases the risk of stroke in postmenopausal women. This Review summarizes the sex differences related to stroke, and the effect of endogenous and exogenous hormones on the cerebrovasculature of the female brain. It also proposes potential research approaches, the results of which could fill in gaps in our knowledge regarding the mechanism of action of estrogen in the brain.

Introduction

Each year, around 700,000 Americans experience a stroke, incurring a total estimated annual cost of $58 billion.[1] Stroke is the third leading cause of death in most countries, and is one of the leading causes of long-term disability.[1] Women have a higher lifetime risk of stroke than men (1 in 5 vs 1 in 6), a statistic that is influenced in part by the longer life expectancy in women.[2] The female population not only carries a higher burden of stroke during their lifespan -- women also account for the majority of stroke deaths.[1] Men typically have a higher incidence of stroke than women up to the age of 75 years, when the incidence in women becomes higher.[1] New data, however, indicate that the incidence of stroke in women in the mid-life years might be increasing. Data from a cohort that was studied from 1999 to 2004 indicated that women aged 45-54 years were around twice as likely to have a stroke as men of the same age.[3] One potential explanation for this finding is that women in this age stratum are transitioning to menopause, a time when cardiovascular risk factors appear or existing risk factors worsen.[4] The incidence of stroke in women might, therefore, be increasing because stroke risk factors go unrecognized or are inadequately treated. Given this surge in stroke prevalence in mid-life women, and the likelihood that stroke risk will continue to increase as women age, from a public health perspective it is

critically important to focus research efforts on understanding stroke in women. The purpose of this Review is to further elucidate sex differences in stroke, to describe the unique risks and benefits of estrogens on the female brain, and to propose future directions of research that might find ways to decrease the burden of stroke in women.

Sex Differences in Stroke

Risk Factors

Cohort studies published over the past decade have clearly and consistently documented that men and women with stroke have different characteristics (Table 1). The women were older and more likely to have atrial fibrillation and hypertension, for example, whereas the men were more likely to have coronary artery disease and to smoke cigarettes.[5-8] Taken together, these studies show that overall, with the exception of diabetes, men and women have different risk factor profiles for stroke (Table 1).

The fact that women tend to be older than men at the time of stroke onset might explain the greater presence in females of risk factors that increase with age, such as atrial fibrillation and hypertension, but there are also sex differences in cardiovascular risk profiles throughout the decades of mid-life. Specifically, National Health and Nutrition Examination Survey (NHANES) data from 1999 to 2004 described not only a surge of stroke incidence in mid-life women, but also a substantial increase in blood pressure, such that by 55-64 years of age, women had a significantly higher average blood pressure than men.[3] In addition, total cholesterol levels in women increased with each decade, whereas cholesterol levels in men tended to remain stable. By 55-64 years of age, women had higher average cholesterol levels than men.[3] Although elevated cholesterol has typically been a weak risk factor for ischemic stroke of all types, it is a notable risk factor for carotid disease. These data indicate that, although stroke is traditionally considered to be a disease of the elderly, especially in women, there should be a new focus on stroke risk factor recognition and management in women during the mid-life decades between 35 and 64 years of age.

The metabolic syndrome represents a cluster of risk factors that are important for future vascular risk, and accumulating evidence indicates that these components differ between men and women. Among individuals who fit the criteria for the metabolic syndrome, women tend to have higher body weight and waist girth, and lower HDL cholesterol, whereas men tend to have higher systolic and diastolic blood pressure.[9] The vascular risk associated with individual components of the metabolic syndrome also differs between the sexes. The Diabetes in Germany study showed that the combination of diabetes, hypertension and low HDL cholesterol was associated with an 11-fold increase in atherosclerotic disease (history of myocardial infarction, coronary revascularization or stroke, or a combination of these factors) in women, whereas men with this cluster of conditions had a fourfold increase in this outcome.[10] In addition, the effect of the metabolic syndrome on early atherosclerosis, as demonstrated by increased carotid intima-media thickness (IMT), is more evident in women than in men.[11] It is important to acknowledge this disparity because carotid IMT can provide an estimate of 10-year cardiovascular risk, including stroke.[12] Taken together, these data indicate that women with the metabolic syndrome have a higher likelihood of subclinical and clinical atherosclerosis than men who have the condition, and would benefit from early targeted prevention strategies.

Atrial fibrillation is a major risk factor for stroke, and, among individuals over the age of 59 years, its prevalence is higher in women than in men.[13] In a registry study of first-ever ischemic stroke patients in Italy, women were 1.6 times more likely to have atrial fibrillation than men (95% CI 1.34-1.83).[13] There are also sex differences related to the risk of thromboembolism and bleeding

complications. In a comprehensive analysis of the Stroke Prevention in Atrial Fibrillation (SPAF) I-III trials, female sex was one of the independent risk factors associated with stroke in individuals with atrial fibrillation who were randomly assigned aspirin.[14] In a separate study of patients with new-onset atrial fibrillation, women were over three times more likely to have a major bleeding complication on warfarin than men.[15] It is important to consider these gender effects when assessing the overall sex differences in ischemic stroke outcomes, because women are more likely than men to have atrial fibrillation over the age of 60 years, women with atrial fibrillation are at least 60% more likely than men to have a stroke, and, in general, strokes that occur because of atrial fibrillation are more severe than those related to other etiologies.[16]

Response to Prevention Therapy

Aspirin is one of the most commonly used and effective antithrombotic therapies for stroke prevention in both men and women.[17,18] For primary prevention of stroke, however, women and men seem to show different cardiovascular profiles for aspirin benefits. A sex-specific meta-analysis of data from over 50,000 women and over 40,000 men enrolled in randomized controlled trials of aspirin for primary prevention reported that, with regard to overall cardiovascular events, both sexes benefited from treatment (12% reduction in cardiovascular events in women vs 14% reduction in men).[19] The overall reduction with aspirin in women was, however, primarily attributable to a 17% reduction in stroke events and no significant effect on myocardial infarction events, whereas in men, the overall reduction was primarily related to a 32% reduction in myocardial infarction events and no significant reduction in stroke events (Table 1).[19] One explanation for this result is that the overall number of events was low in these primary prevention trials. Also, there were more stroke events in women and more myocardial infarction events in men, thereby limiting the evaluation of effect on low-frequency events in each group. Despite the apparent differential effect of aspirin on particular types of vascular events in men and women, the overall cardiovascular benefit was similar.[19] Further research on potential sex differences in the response to primary prevention therapy is clearly needed.

Substantial sex differences in response to secondary prevention of symptomatic intracranial disease have also recently been recognized. The Warfarin-Aspirin Symptomatic Intracranial Disease (WASID) study enrolled 350 men and 219 women with stroke (mean age of both groups approximately 63 years).[20] There were baseline differences by sex, such that the women were more likely than the men to be African American (38% vs 26%), whereas the men were more likely to be white (63% vs 51%). The women were also more likely than the men to be widowed, separated or divorced, or living alone. The primary end point for this analysis was brain hemorrhage, recurrent ischemic stroke, or death from vascular causes other than stroke. Strikingly, the event rate was 28% for women and 16.6% for men over the 2 years of follow-up. The adjusted hazard ratio for ischemic stroke was 1.85 (95% CI 1.14-3.01) for women, even after accounting for baseline differences between the women and the men.[20] This analysis reinforces the hypothesis that women carry a substantial proportion of the burden of stroke. In the absence of effective therapies for intracranial disease, primary prevention of risk factors should be the top priority.

Response to Treatment

Recent studies have indicated that men and women respond differently to acute stroke treatment with thrombolysis. For example, a pooled analysis of individuals from three trials in which acute stroke was treated with tissue plasminogen activator (tPA) showed that women had a better outcome at 90 days (good outcome defined as a modified Rankin Score ≤1) after tPA treatment compared with the placebo group than did men (Table 1).[21] Adjusting for the time between stroke symptom onset and treatment did not appreciably change the results. One interpretation of these results is that women treated with placebo had significantly worse outcome at 90 days than men

receiving placebo, indicating that women who are not treated with tPA have a poor outlook. Similarly, a randomized trial of intra-arterial urokinase for middle cerebral artery occlusions found that women showed greater benefits from recanalization than did men.[22] An observational study in Canada of consecutive stroke patients treated with intravenous tPA confirmed that female sex was associated with major neurological improvement.[23] In this study, women were 2.4 times more likely than men to have an NIH Stroke Scale (NIHSS) score of 0 or 1 or an improvement of ≤8 points on the NIHSS in the first 24 hours after stroke (odds ratio [OR] 2.40, 95% CI 1.12-5.14).[23]

Not all studies have shown a significant sex-related difference in response to acute stroke treatment. For example, a secondary analysis of a clinical trial that was originally designed to test the efficacy of a potential neuroprotectant therapy showed that intravenous tPA did not reverse the tendency for women to have worse outcomes than men.[24] Rapid improvement in response to tPA in the first hour of treatment was, however, an exclusion criterion for participation in the neuroprotectant trial, which might explain the negative findings. In addition, an observational study of consecutive patients treated with intra-arterial thrombolysis failed to demonstrate a significant difference in outcomes on the basis of sex.[25] Further studies will be required to determine whether there are sex differences in the response to acute stroke treatments of all types.

Outcomes

In general, women have worse outcomes after stroke than men. Of all stroke deaths, 61% occur in women.[1] Among individuals who survive stroke, women are more likely than men to be institutionalized,[5,6,8,26,27] and they tend to have worse prestroke and poststroke disability.[5,7,8,27] In a study of functional outcomes after stroke in Norway, men were three times more likely to achieve a high score on the Barthel Index (an activities of daily living scale) than women, and women were six times more likely to be institutionalized at 1 year than men.[28] Women are also twice as likely as men to develop poststroke depression.[29]

In addition, there are race-ethnic differences in outcomes after stroke. For example, in the National Institute for Neurological Disorders and Stroke (NINDS) tPA trial, African American women had the highest 1-year mortality when compared with white men, white women and African American men.[30] It might, therefore, be inappropriate to generalize outcomes to all women if only one race-ethnic group is represented.

The reasons for poor outcomes after stroke in women are likely to be multifactorial. Women might be older at the time of stroke than are men, and, therefore, more likely to have prestroke disabilities and comorbidities.[5,6,8,27] Women might also lack social support because they are widowed and living alone at the time of the stroke, creating delays in presentation for effective therapies such as tPA. One study has shown that on arrival at the hospital, diagnostic tests such as echocardiograms and carotid evaluations are less likely to be ordered for women than for men, and important etiological information might, therefore, be missed.[31]

Another explanation for worse outcomes in women than men might be related to anatomical and cerebrovascular mechanisms. There are recognized differences in the anatomical organization of brain functions in men and women. For instance, the representation of language is more bilateral in women than in men.[32] Most studies have not shown sex differences in the incidence of aphasia,[33] although one study reported that women were more likely than men to present with aphasia as a symptom of stroke.[7] This effect might be more closely related to the site and the type of stroke than to sex,[33] as women are more likely to have atrial fibrillation and cardioembolic stroke. One cerebrovascular mechanism that could affect recovery is vasomotor reserve -- the capacity of the brain to maintain adequate cerebral blood flow, particularly distal to a narrowed vessel. It has been shown that poor vasomotor reserve is associated with a substantial risk of

stroke in the setting of severe carotid stenosis.[34] There is a paucity of data regarding sex differences in vasomotor reserve, but one study showed that postmenopausal women had reduced vasomotor reserve compared with premenopausal women and age-matched men.[35] A reduction in collateral blood flow to the ischemic penumbra might, therefore, influence recovery and outcome after stroke. No rigorous study of sex differences in vasomotor reserve in relation to stroke recovery has been published yet.

There might also be sex differences within the microenvironment of the neurovascular unit (NVU), a conceptual model of the interactions and signaling among neurons, endothelial cells and glia.[36,37] Examining the interactions within this microenvironment could help to explain the differences between women and men with regard to responses to vascular stress, and to acute stroke treatments and their complications (e.g. hemorrhagic transformation), and with regard to recovery of function after stroke. Investigation of the effects of exogenous and endogenous estrogen on the NVU should provide insight into the sex differences observed in humans with stroke.

Endogenous Hormones and Cerebrovascular Disease

Menstrual Cycle

The menstrual cycle in the human female reflects changing levels of estradiol during ovulatory and nonovulatory phases. Estradiol has potent vasodilatory effects on the endothelium; for example, brachial artery flow-mediated dilation in response to hyperemia has been shown to positively correlate with the levels of estradiol throughout the menstrual cycle in young healthy women.[38] Similarly, cerebral blood flow velocities and vasomotor reactivity, as measured with transcranial Doppler (TCD) ultrasound, vary in response to physiologic levels of estradiol. Diomedi *et al.* studied TCD velocities and vasomotor reactivity (measured using the breath-holding index) during the menstrual and ovulatory phases of the menstrual cycle in healthy women.[39] Compared with the menstrual phase, there was an increased vasodilatory response to hypercapnia during ovulation, which corresponds to the highest levels of estradiol. Consistent with the estradiol effect, there was no difference in cerebrovascular reactivity between women in the menstrual phase of the cycle and age-matched healthy men.[39]

A relationship between supraphysiologic estradiol levels and cerebral hemodynamics has been demonstrated in women undergoing controlled ovarian hyperstimulation.[40] Compared with the period of lowest estradiol levels that occur after leuprolide acetate administration, the peak estradiol levels resulting from human menopausal gonadotropin treatment corresponded to a significantly higher pulsatility index in the bilateral middle cerebral arteries. There was a significant correlation between the middle cerebral artery velocities and the natural log of estradiol levels (r = 0.93; P <0.0001). Physiologic and supraphysiologic levels of estradiol might, therefore, have substantial influences on cerebral hemodynamics in young childbearing women. Owing to the small numbers of individuals studied, however, the relationship between these blood flow studies and stroke risk is uncertain.

Pregnancy

Pregnancy represents a state when endogenous estrogen levels are continuously elevated, dropping precipitously in the first few days postpartum. Interestingly, most cerebrovascular complications occur at delivery or postpartum. For example, one study showed that the risk of ischemic stroke or intracerebral hemorrhage (ICH) during the 6 weeks after delivery was more than 12 times that in nonpregnant women, but there was no significant risk of either type of event throughout the rest of pregnancy.[41] Another study, from Sweden, reported that most venous or arterial events actually occurred in the period from 2 days before to 1 day after delivery.[42] As a result of natural selection against fatal hemorrhage at delivery, pregnancy represents a relative

hypercoagulable state, which might explain the increased risk of thromboembolic complications. This fact does not, however, explain the increased risk of ICH, which occurs almost exclusively postpartum.[43] The reasons for the postpartum predilection for both ischemic stroke and ICH are poorly understood, but it has been proposed that a large decrease in blood volume, rapid fluctuations in hormone levels, or vessel wall changes, can profoundly influence cerebral hemodynamics.[41]

The causes of stroke during pregnancy or the puerperium are multiple and heterogeneous.[41,44] Although many causes (such as cervical artery dissection) cannot be anticipated before the event, several predictive risk factors have been identified in multiple cohorts (Table 2). These include maternal age greater than 35 years, African American race, migraine headaches, thrombophilia, systemic lupus erythematosus, sickle cell disease, pre-eclampsia-eclampsia, pre-existing hypertension, and heart disease.[45] Of these risk factors, pre-eclampsia -- one of the hypertensive disorders of pregnancy -- could be the most important because it increases the risk of multiple types of cerebrovascular complications. Pregnant women with pre-eclampsia-eclampsia were shown have a more than tenfold higher risk of pregnancy-related ICH compared with women without pre-eclampsia-eclampsia.[43] Pre-eclampsia-eclampsia has also been associated with vasculopathies that lead to vasogenic edema, or to vasoconstriction syndromes that can result in ischemic stroke.[46,47] Women with pre-eclampsia have higher pulse wave velocities in peripheral arteries, indicating arterial stiffness.[48,49] In addition, cerebral blood flow velocities measured with TCD by Demarin and colleagues were significantly elevated throughout the third trimester in women with pre-eclampsia compared with age-matched controls, and normalization of velocities occurred 2 months postpartum.[50] The routine use of TCD monitoring in women at high risk for pre-eclampsia might predict not only the severity of pre-eclampsia, but also the risk of cerebrovascular complications.[50] Beyond pregnancy, pre-eclampsia also increases the risk of chronic hypertension, as well as stroke and cardiovascular disease later in life.[51-55]

Menopause

Estradiol levels decrease during natural menopause, and then decline further following menopause until reaching a plateau after 1-3 years. Compared with the decline in estradiol, testosterone levels decrease more gradually during this transition, leading to a relative androgen excess.[56] These changes are important because androgens and estrogens have opposite effects on vascular risk. Estradiol improves vasodilation and arterial compliance by decreasing vascular tone, thus increasing cerebral blood flow.[57] Estrogens protect cerebral endothelial cells by increasing the efficiency of mitochondrial energy production while decreasing free radical production and enhancing endothelial cell survival.[57] Other beneficial effects of estrogens include anti-inflammatory and antioxidant actions, and the improvement of atherogenic lipid profiles by lowering total and LDL cholesterol, and lipoprotein(a), and raising HDL cholesterol.[58,59] Conversely, androgens have a detrimental effect on cerebral blood vessels by increasing arterial tone, decreasing levels of HDL to cause a pro-atherogenic profile, and increasing levels of triglycerides and LDL and total cholesterol.[56,57]

In view of the physiological changes that result from estradiol depletion and relative androgen excess, it is not surprising that women develop changes in cardiovascular risk factors during menopause. In the Healthy Women Study, postmenopausal women had significantly higher LDL cholesterol and triglycerides, lower HDL cholesterol, and higher fasting glucose and blood pressure, compared with premenopausal women.[4] Along with the change in body fat distribution towards abdominal obesity, the lipid, blood pressure and glucose profiles in postmenopausal women are consistent with those seen in the metabolic syndrome.[60] There is also evidence of an increase in subclinical disease between the premenopausal and postmenopausal states, as indicated by carotid atherosclerosis (measured by carotid IMT).[61] Vasomotor reactivity declines

markedly in postmenopausal women compared with age-matched men and premenopausal women.[35] The cumulative effect of the increase in cardiovascular risk seen in postmenopausal women was recently demonstrated in an analysis of the NHANES data.[3] This analysis revealed that women show a substantially larger increase in stroke prevalence with advancing age than do men, as well as a greater relative increase in blood pressure and cholesterol. These data emphasize the importance of targeting women who are transitioning through menopause for risk factor recognition and management.

Exogenous hormones have been used for contraception and postmenopausal hormone replacement for over 40 years. The risk of cerebrovascular disease seems to increase with the use of synthetic estrogens, newer generations of progestins (especially second generation), and formulations containing higher doses of the estrogen component. It is, however, important to discuss oral contraceptive formulations separately to those used for postmenopausal hormone replacement.

Oral Contraceptive Pills

Oral contraceptive pills (OCPs) first became available in the 1960s. An epidemiology study, published in 1987, examined cerebrovascular deaths in males and females aged 15-44 years during the time periods before and after OCPs became available in Denmark.[62] There was a trend towards an increase in the female:male death from ischemic stroke ratios (but not subarachnoid hemorrhage or ICH) in the 13 years following OCP availability compared with the 13 years before OCP availability, especially in women aged 15-34 years. The authors of this study suggested that OCP use might have partially explained this trend, but cause and effect could not be established.[62]

Since 1987, multiple case-control and cohort studies have examined the effect of OCPs on stroke risk. This relationship has been summarized in three different meta-analyses. The first analysis, published in 2000, examined 16 studies, and reported that the cumulative relative risk (RR) of ischemic stroke with OCP use was 2.75 (95% CI 2.24-3.38).[63] Lower estrogen doses were associated with a lower risk of ischemic stroke than higher doses, but all dosages significantly elevated the risk. In addition, studies that used hospital controls, and those that did not control for smoking, showed higher RRs. A second meta-analysis, published in 2004, estimated cumulative RR ratios from 20 studies (16 case-control and 4 cohort studies).[64] Unlike the previous meta-analysis, the authors reported that the summary RR depended on the study design. Case-control studies indicated that OCP use was associated with an elevated risk of stroke (OR 2.13, 95% CI 1.59-2.86), but cohort studies did not reveal a significantly increased risk (OR 0.95, 95% CI 0.51-1.78). On the basis of the thromboembolic risk associated with high estrogen doses, OCPs should primarily increase the risk of ischemic stroke. There are caveats to the summary OR from the cohort studies, however, because out of the four cohort studies, only one specifically separated ischemic strokes from hemorrhagic strokes, and the other three studied either hemorrhagic stroke alone or ischemic and hemorrhagic stroke combined. A third meta-analysis, published in 2005, showed that the summary OR for ischemic stroke was 2.12 (95% CI 1.56-2.86), compared with 1.84 (95% CI 1.38-2.44) for myocardial infarction.[65] This analysis also reported a significantly higher risk of both ischemic stroke and myocardial infarction in women taking second-generation OCPs than in those not taking OCPs, and third-generation OCPs increased the risk of ischemic stroke but not myocardial infarction. It should be emphasized that the absolute risk of stroke is still very small; there is about one additional ischemic stroke per year per 24,000 women using OCPs.[64]

Postmenopausal Hormone Therapy

In 1995, an estimated 38% of women in the US were using postmenopausal hormone therapy.[66] This high proportion of use was attributed in part to the cardiovascular and hip fracture protection

benefits reported in observational studies. These benefits outweighed the potential risks of uterine cancer, as reported in a landmark meta-analysis in 1992.[67] Randomized controlled trials have shown that hormone therapy does not protect against stroke in the context of secondary prevention, and seems to increase risk in the context of primary prevention. The Heart and Estrogen/progestin Replacement Study (HERS) demonstrated that women with established coronary disease who were randomly assigned to conjugated equine estrogen-medroxyprogesterone acetate (CEE-MPA) did not have fewer recurrent coronary artery disease or stroke events than women receiving placebo.[68,69] Similarly, the Women's Estrogen for Stroke Trial (WEST) showed that women with minor stroke or transient ischemic attack did not have fewer recurrent events after treatment with 17ß-estradiol than after treatment with placebo.[70] In fact, in a *post hoc* analysis, there was a statistically significant increase in strokes in the first 6 months after randomization to this drug, demonstrating an early risk. Finally, the Women's Health Initiative (WHI) study showed that relatively healthy postmenopausal women randomly allocated to CEE-MPA (or CEE alone in those with hysterectomies) had a 40% increased risk of stroke during follow-up.[71,72] Women using postmenopausal hormone therapy also had an increased risk of venous thromboembolism, coronary artery disease events (with CEE-MPA, but not with CEE alone),[71,72] probable dementia, mild cognitive impairment,[73] and breast cancer.[71] Not surprisingly, the prescription of hormone therapy has decreased markedly in the years following the initial WHI publication.[74] Currently, hormone therapy is indicated only for the treatment of menopausal vasomotor symptoms and vaginal dryness.[75]

Despite this convincing clinical trial evidence, the reasons for the increased risk of stroke in the WHI are poorly understood. Multiple subgroup analyses of both arms of the study failed to identify specific characteristics in the women at particularly low or high risk of stroke with hormone therapy.[76,77] One of the primary criticisms of the WHI was that the majority of participants were over the age of 60 years; in other words, they were on average 10 years postmenopausal and past the usual age for treatment of vasomotor symptoms.[78] The relationship between time since menopause and occurrence of coronary heart disease (CHD) or stroke events was analyzed in the CEE-MPA and CEE-alone arms of the trial.[79] Interestingly, there was a trend towards a lower risk of CHD in women who initiated hormone therapy closer to menopause, whereas occurrence of stroke events was not affected by the time since menopause. Again, there is no clear explanation for why estrogen might have provided some early benefit in reduction of heart disease in mid-life, but no benefit with respect to risk of stroke.

A subsequent analysis focusing on the CEE-alone arm of the WHI showed that hormone therapy led to a reduction in the coronary artery calcium score, a measure of subclinical coronary artery disease.[80] This finding might help to explain why there was no increased risk of CHD in the CEE-alone arm of the study. These results are, however, likely to be specific to CHD and cannot be extended to cerebrovascular disease, as there was a significant increase in stroke events in this trial.[76] It is important, therefore, to emphasize that future analyses of the WHI should be focused on understanding how hormone therapy -- particularly the estrogen component -- increased the risk of stroke regardless of hysterectomy status.

Although the WHI provides crucial data on the associations between CEE, alone or in combination with MPA, and multiple vascular diseases, it still leaves many unanswered questions. One major gap in the analyses of stroke events in the WHI is the fact that initial stroke severity was not measured using standardized neurological impairment scales such as the NIHSS.[81] In addition, none of the outcomes measured after hospitalization reflected recovery and disability, including stroke severity and functional status. This fact is important because many women recover from stroke completely, whereas others are left with substantial disability, and it is important to determine whether the outcomes of women in the trial correlated with the use of hormone therapy at the time of stroke.

Advancing the Study of Stroke in Women

A multidisciplinary working group convened in August 2005 to further explore the unanswered questions from the WHI and the effect of estrogen on stroke and cardiovascular disease. The Advancing the Study of Stroke in Women workshop, sponsored in part by the NINDS, aimed to provide specific recommendations for future clinical and basic-science research that will improve our understanding of the risks and benefits of estrogens with respect to stroke and vascular disease, explain sex differences in stroke outcomes and responses to stroke treatment, and highlight wide gaps in knowledge regarding stroke in high risk populations, such as pregnant or African American women.[36] One of the critical gaps in basic-science knowledge is whether the NVU, which can be used as a conceptual model of cerebral ischemia and the response to specific treatments, differs by sex. One initial step is to test all new acute stroke therapies in both male and female animal models to ensure that the treatment responses are the same, and, if not, to determine at the molecular level the nature of the differences. There should also be a continued basic-science effort to determine how sex steroids and their mimetics might act as protectants in specific models of neurovascular injury.[36]

One protective action of estrogen is its influence on inflammatory and immune responses in the brain. Microglia are an important component of the NVU, as these cells represent the mononuclear phagocyte system in the brain and are in close proximity to the cerebral blood vessels and the blood-brain barrier.[82] Estrogen has anti-inflammatory effects in the brain that are specifically mediated by glial cells, such as the microglia. Through the ERα and ERß estrogen receptors located in microglia, estrogen downregulates proinflammatory innate immune responses.[83] The anti-inflammatory actions of estrogen vary, however, depending on apolipoprotein E (*APOE*) genotype. In a recent study by Brown and co-workers, estrogen modulated the expected anti-inflammatory effects in *APOE3* targeted replacement mice, but did not suppress inflammation to the same extent in *APOE4* mice.[84] If these data can be extrapolated to humans, postmenopausal women who have the *APOE4* genotype and are treated with hormone therapy might not benefit from the anti-inflammatory actions of estrogen in the brain. In view of the fact that cerebrovascular disease and atherosclerosis are inflammatory conditions, genetic variability might contribute to the unexplained risk of ischemic stroke associated with hormone therapy in primarily healthy women in the WHI. Indeed, in a study of elderly women with Alzheimer's disease who were using estrogen, those with the *APOE4* genotype did not receive the beneficial slowing of cognitive decline from estrogen use that was seen with *APOE4*-negative genotypes.[85] In addition, among *APOE4*-positive women, there was no difference in internal carotid artery wall IMT between those who used estrogen and those who did not, whereas *APOE4*-negative women who used estrogen had significantly lower IMT than non-users. This study indicates that there is an interaction between *APOE* status and estrogen use with regard to both neuronal function and progression of carotid atherosclerosis. Further basic-science and clinical research efforts should be focused on determining the gene-environment interactions that relate to estrogens, and how this knowledge could help target estrogen therapy to women who will benefit.[36]

The working group also recommended several specific clinical research imperatives. The population targeted for clinical studies of the interactions between estrogen and stroke should be limited to those women who are candidates for hormone therapy during perimenopause, early postmenopause, or postsurgical menopause. Methods to distinguish between women at high risk of stroke with hormone therapy, and those who might benefit from hormone therapy, need to be developed. Candidate genetic, inflammatory or thrombosis markers should be investigated for this purpose. In addition, hormone therapy formulations other than those used in the WHI need to be investigated for stroke risk, and other surrogate measures of subclinical disease might be required because stroke as an outcome measure might be too rare to study with short-term follow-up.[36]

Conclusions and Future Research Opportunities

Besides the clinical and basic-science research opportunities discussed at the Advancing the Study of Stroke in Women Workshop, translational approaches will be required to further the study of the relationships between sex, estrogen and stroke. For example, peripheral vessel endothelial responses (e.g. brachial artery flow-mediated dilation) can be measured in the setting of various polymorphisms, different levels of endogenous hormones, or treatment with exogenous hormones. Cerebrovascular responses in these subgroups can be measured with middle cerebral velocity changes in response to a carbon dioxide challenge to determine cerebral vasomotor reserve.[34] In addition, use of surrogate outcomes such as IMT of the carotid artery -- which predicts 10-year cardiovascular risk[12] and can be measured in response to various stroke prevention treatments -- can enable therapies to be focused on younger women, who have an overall low stroke event rate but are accumulating risk factors.[86]

Animal models of focal and global cerebral ischemia have shown that estrogens have a beneficial effect on all the individual components of the NVU.[37] Clinical trials of exogenous estrogen treatment in postmenopausal women have, however, shown either no benefit or an increased risk of stroke when compared with placebo.[69,70,76] Explaining the gap between the beneficial effects of exogenous estrogens in the brain demonstrated in animal studies and the risk of stroke in response to estrogens in clinical trials will require investigation of the NVU as a whole in both males and females, taking into account age, child-bearing status, vascular risk factors, endogenous levels of sex hormones, and genetic polymorphisms.[36]

References

1. Thom T *et al.* (2006) Heart disease and stroke statistics -- 2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 113: e85-e151.
2. Seshadri S *et al.* (2006) The lifetime risk of stroke: estimates from the Framingham Study. Stroke 37: 345-350.
3. Towfighi A *et al.* (2007) A midlife stroke surge among women in the United States. Neurology [doi: 10.1212/1201.wnl.0000268491.0000289956.c0000268492].

4. Matthews KA *et al.* (2001) Changes in cardiovascular risk factors during the perimenopause and postmenopause and carotid artery atherosclerosis in healthy women. Stroke 32: 1104-1111.
5. Di Carlo A *et al.* (2003) Sex differences in the clinical presentation, resource use, and 3-month outcome of acute stroke in Europe: data from a Multicenter Multinational Hospital-Based Registry. Stroke 34: 1114-1119.
6. Holroyd-Leduc JM *et al.* (2000) Sex differences and similarities in the management and outcome of stroke patients. Stroke 31: 1833-1837.
7. Roquer J *et al.* (2003) Sex differences in first-ever acute stroke. Stroke 34: 1581-1585.
8. Kapral MK *et al.* (2005) Sex differences in stroke care and outcomes: results from the registry of the Canadian Stroke Network. Stroke 36: 809-814.
9. Dallongeville J *et al.* (2004) The association of metabolic disorders with the metabolic syndrome is different in men and women. Ann Nutr Metab 48: 43-50.
10. Hanefeld M *et al.* (2007) Impact of the individual components of the metabolic syndrome and their different combinations on the prevalence of atherosclerotic vascular disease in type 2 diabetes: the Diabetes in Germany (DIG) study. Cardiovasc Diabetol 6: e13.
11. Iglseder B *et al.* (2005) The metabolic syndrome is a stronger risk factor for early carotid atherosclerosis in women than in men. Stroke 36: 1212-1217.
12. Howard G *et al.* for the ARIC Investigators (1993) Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. Stroke 24: 1297-1304.
13. Marini C *et al.* (2005) Contribution of atrial fibrillation to incidence and outcome of ischemic stroke: results from a population-based study. Stroke 36: 1115-1119.
14. Hart RG *et al.* (1999) Factors associated with ischemic stroke during aspirin therapy in atrial fibrillation: analysis of 2012 participants in the SPAF I-III clinical trials. Stroke 30: 1223-1229.
15. Humphries K *et al.* (2001) New-onset atrial fibrillation: sex differences in presentation, treatment, and outcome. Circulation 103: 2365-2370.
16. Lin H-J *et al.* (1996) Stroke severity in atrial fibrillation: the Framingham study. Stroke 27: 1760-1764.
17. Antithrombotic Trialists' Collaboration (2002) Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 324: 71-86.
18. Antiplatelet Trialists' Collaboration (1994) Collaborative overview of randomised trials of antiplatelet therapy -- I. Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. BMJ 308: 81-106.
19. Berger J *et al.* (2006) Aspirin for the primary prevention of cardiovascular events in women and men: a sex-specific meta-analysis of randomized controlled trials. JAMA 295: 306-313.
20. Williams J *et al.* (2007) Gender differences in outcomes among patients with symptomatic intracranial arterial stenosis. Stroke 38: 2055-2062.
21. Kent DM *et al.* (2005) Sex-based differences in response to recombinant tissue plasminogen activator in acute ischemic stroke: a pooled analysis of randomized clinical trials. Stroke 36: 62-65.
22. Hill MD *et al.* (2006) Sex-based differences in the effect of intra-arterial treatment of stroke: analysis of the PROACT-2 study. Stroke 37: 2322-2325.
23. Saposnik G *et al.* (2005) Predictors of major neurologic improvement after thrombolysis in acute stroke. Neurology 65: 1169-1174.
24. Elkind M *et al.* (2007) Sex as a predictor of outcomes in patients treated with thrombolysis for acute stroke. Neurology 68: 842-848.

25. Shah S *et al.* (2006) Influence of gender on outcomes after intra-arterial thrombolysis for acute ischemic stroke. Neurology 66: 1745-1746.
26. Kelly-Hayes M *et al.* (2003) The influence of gender and age on disability following ischemic stroke: the Framingham study. J Stroke Cerebrovasc Diseases 12: 119-126.
27. Glader EL *et al.* for the Riks-Stroke Collaboration (2003) Sex differences in management and outcome after stroke: a Swedish national perspective. Stroke 34: 1970-1975.
28. Wyller T *et al.* (1997) Are there gender differences in functional outcome after stroke? Clin Rehabil 11: 171-179.
29. Paradiso S and Robinson R (1998) Gender differences in poststroke depression. J Neuropsychiatry Clin Neurosci 10: 41-47.
30. Qureshi A *et al.* (2006) African American women have poor long-term survival following ischemic stroke. Neurology 67: 1623-1629.
31. Smith MA *et al.* (2005) Gender comparisons of diagnostic evaluation for ischemic stroke patients. Neurology 65: 855-858.
32. Shaywitz BA *et al.* (1995) Sex differences in the functional organization of the brain for language. Nature 373: 607-609.
33. Hier D *et al.* (1994) Gender and aphasia in the Stroke Data Bank. Brain Lang 47: 155-167.
34. Müller M and Schimrigk K (1996) Vasomotor reactivity and pattern of collateral blood flow in severe occlusive carotid artery disease Stroke 27: 296-299.
35. Matteis M *et al.* (1998) Age and sex differences in cerebral hemodynamics: a transcranial Doppler study. Stroke 29: 963-967.
36. Bushnell CD *et al.* (2006) Advancing the Study of Stroke in Women: summary and recommendations for future research from an NINDS-sponsored multidisciplinary working group. Stroke 37: 2387-2399.
37. Yang S *et al.* (2005) Estrogens as protectants of the neurovascular unit against ischemic stroke. Curr Drug Targets CNS Neurol Disord 4: 169-177.
38. Hashimoto M *et al.* (1995) Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. Circulation 92: 3431-3435.
39. Diomedi M *et al.* (2001) Influence of physiologic oscillation of estrogens on cerebral hemodynamics. J Neurol Sci 185: 49-53.
40. Shamma FN *et al.* (1992) Middle cerebral artery blood velocity during controlled ovarian hyperstimulation. Fertil Steril 57: 1022-1025.
41. Kittner SJ *et al.* (1996) Pregnancy and the risk of stroke. N Engl J Med 335: 768-774.
42. Ros HS *et al.* (2001) Increased risks of circulatory diseases in late pregnancy and puerperium. Epidemiology 12: 456-460.
43. Bateman B *et al.* (2006) Intracerebral hemorrhage in pregnancy: frequency, risk factors, and outcome. Neurology 67: 424-429.
44. Sharshar T *et al.* for the Stroke in Pregnancy Study Group (1995) Incidence and causes of strokes associated with pregnancy and puerperium: a study in public hospitals of Île de France. Stroke 26: 930-936.
45. James A *et al.* (2005) Incidence and risk factors for stroke in pregnancy and the puerperium. Obstet Gynecol 106: 509-516.
46. Singhal AB (2004) Postpartum angiopathy with reversible posterior leukoencephalopathy. Arch Neurol 61: 411-416.
47. Zunker P *et al.* (1995) Cerebral hemodynamics in pre-eclampsia/eclampsia syndrome. Ultrasound Obstet Gynecol 6: 411-415.
48. Elvan-Taspinar A *et al.* (2004) Central hemodynamics of hypertensive disorders in pregnancy. Am J Hypertens 17: 941-946.
49. Ronnback M *et al.* (2005) Pulse wave reflection in currently and previously preeclamptic women. Hypertens Pregnancy 24: 171-180.

50. Demarin V *et al.* (1997) Maternal cerebral circulation in normal and abnormal pregnancies. Acta Obstet Gynecol Scand 76: 619-624.
51. Chireau MV *et al.* (2005) Pregnancy complications are associated with stroke risk later in life. Ann Neurol 58: S41.
52. Ray J *et al.* (2005) Cardiovascular health after maternal placental syndromes (CHAMPS): population-based retrospective cohort study. Lancet 366: 1797-1803.
53. Wilson BJ *et al.* (2003) Hypertensive diseases of pregnancy and risk of hypertension and stroke in later life: results from cohort study. BMJ 326: 845-852.
54. Brown DW *et al.* (2006) Preeclampsia and the risk of ischemic stroke among young women: results from the Stroke Prevention in Young Women Study. Stroke 37: 1055-1059.
55. Smith GC *et al.* (2001) Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. Lancet 357: 2002-2006.
56. Liu Y *et al.* (2001) Relative androgen excess and increased cardiovascular risk after menopause: a hypothesized relation. Am J Epidemiol 154: 489-494.
57. Krause D *et al.* (2006) The influence of sex steroid hormones on cerebrovascular function. J Appl Physiol 101: 1252-1261.
58. The Writing Group for the PEPI Trial (1995) Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. JAMA 273: 199-208.
59. Haines C *et al.* (1996) Effect of oral estradiol on Lp(a) and other lipoproteins in postmenopausal women. Arch Intern Med 156: 866-872.
60. Spencer C *et al.* (1997) Is there a menopausal metabolic syndrome? Gynecol Endocrinol 11: 341-355.
61. Sutton-Tyrrell K *et al.* (1998) Carotid atherosclerosis in premenopausal and postmenopausal women and its association with risk factors measured after menopause. Stroke 29: 1116-1121.
62. Lidegaard O (1987) Cerebrovascular deaths before and after the appearance of oral contraceptives. Acta Neurol Scand 75: 427-433.
63. Gillum LA *et al.* (2000) Ischemic stroke risk with oral contraceptives: a meta-analysis. JAMA 284: 72-78.
64. Chan W-S *et al.* (2004) Risk of stroke in women exposed to low-dose oral contraceptives: a critical evaluation of the evidence. Arch Intern Med 164: 741-747.
65. Baillargeon J-P *et al.* (2005) Association between the current use of low-dose oral contraceptives and cardiovascular arterial disease: a meta-analysis. J Clin Endocrinol Metab 90: 3863-3870.
66. Keating N *et al.* (1999) Use of hormone replacement therapy by postmenopausal women in the United States. Ann Intern Med 130: 545-553.
67. Grady D *et al.* (1992) Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Internal Med 117: 1016-1037.
68. Hulley S *et al.* for the Heart and Estrogen/progestin Replacement Study (HERS) Research Group (1998) Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 280: 605-613.
69. Simon JA *et al.* (2001) Postmenopausal hormone therapy and risk of stroke: the Heart and Estrogen-progestin Replacement Study (HERS). Circulation 103: 638-642.
70. Viscoli CM *et al.* (2001) A clinical trial of estrogen-replacement therapy after ischemic stroke. N Engl J Med 345: 1243-1249.
71. Writing Group for the Women's Health Initiative Investigators (2002) Risks and benefits of estrogen plus progestin in healthy post-menopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 288: 321-333.

72. The Women's Health Initiative Steering Committee (2004) Effects of conjugated equine estrogen in postmenopausal women with hysterectomy. JAMA 291: 1701-1712.
73. Rapp SR *et al.* (2003) Effect of estrogen plus progestin on global cognitive function in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 289: 2663-2672.
74. Hersh AL *et al.* (2004) National use of postmenopausal hormone therapy: annual trends and response to recent evidence. JAMA 291: 47-53.
75. Barrett-Connor E *et al.* (2005) The rise and fall of menopausal hormone therapy. Annu Rev Public Health 26: 115-140.
76. Hendrix SL *et al.* (2006) Effects of conjugated equine estrogen on stroke in the Women's Health Initiative. Circulation 113: 2425-2434.
77. Wassertheil-Smoller S *et al.* (2003) Effect of estrogen plus progestin on stroke in postmenopausal women: the Women's Health Initiative: a randomized trial. JAMA 289: 2673-2684.
78. Klaiber EL *et al.* (2005) A critique of the Women's Health Initiative hormone therapy study. Fertil Steril 84: 1589-1601.
79. Roussouw J *et al.* (2007) Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA 297: 1465-1477.
80. Manson JE *et al.* (2007) Estrogen therapy and coronary-artery calcification. N Engl J Med 356: 2591-2602.
81. Brott T *et al.* (1989) Measurements of acute cerebral infarction: a clinical examination scale. Stroke 20: 864-870.
82. Colton C *et al.* (2005) Sex steroids, *APOE* genotype and the innate immune system. Neurobiol Aging 26: 363-371.
83. Vegeto E *et al.* (2001) Estrogen prevents the LPS-induced inflammatory response in microglia. J Neurosci 21: 1809-1818.
84. Brown C *et al.* (2007) The *APOE4* genotype alters the response of microglia and macrophages to 17ß-estradiol. Neurobiol Aging [doi: 10.1016/j.neurobiolaging.2007.04.018].
85. Yaffe K *et al.* (2000) Estrogen use, APOE and cognitive decline: evidence of gene-environment interaction. Neurology 54: 1949-1954.
86. Schott L *et al.* (2004) Segment-specific effects of cardiovascular risk factors on carotid artery intima-medial thickness in women at midlife. Arterioscler Thromb Vasc Biol 24: 1951-1956.
87. Carandang R *et al.* (2006) Trends in incidence, lifetime risk, severity, and 30-day mortality of stroke over the past 50 years. JAMA 296: 2939-2946.
88. Kolominsky-Rabas P *et al.* (2001) Epidemiology of ischemic stroke subtypes according to TOAST criteria: incidence, recurrence, and long-term survival in ischemic stroke subtypes: a population-based study. Stroke 32: 2735-2740.
89. Jaigobin C and Silver FL (2000) Stroke and pregnancy. Stroke 31: 2948-2951.
90. Lanska DJ and Kryscio RJ (1998) Stroke and intracranial venous thrombosis during pregnancy and puerperium. Neurology 51: 1622-1628.
91. Lanska DJ and Kryscio RJ (2000) Risk factors for peripartum and postpartum stroke and intracranial venous thrombosis. Stroke 31: 1274-1282.
92. Jeng J-S *et al.* (2004) Stroke in women of reproductive age: comparison between stroke related and unrelated to pregnancy. J Neurol Sci 221: 25-29.