Advancing the Study of Stroke in Women

Summary and Recommendations for Future Research From an NINDS-Sponsored Multidisciplinary Working Group

Cheryl D. Bushnell, MD, MHS; Patricia Hurn, PhD; Carol Colton, PhD; Virginia M. Miller, PhD; Gregory del Zoppo, MD, MS; Mitchell S.V. Elkind, MD; Barney Stern, MD; David Herrington, MD; Gwendolyn Ford-Lynch, MD; Philip Gorelick, MD, MPH; Andra James, MD, MPH; Candice M. Brown, PhD; Emily Choi, MD; Paul Bray, MD; L. Kristin Newby, MD, MHS; Larry B. Goldstein, MD; James Simpkins, PhD

Background and Purpose—Women have poorer outcomes from stroke than men. Women also have risk factors that are unique, including pregnancy and hormone therapy. Hormone therapy for postmenopausal replacement increased the risk of ischemic stroke according to results of the Women’s Health Initiative clinical trials. Based on the current understanding of the mechanisms of action of estrogen, the reasons for this increased risk are uncertain. One method to better understand the reasons for this increased risk is to re-evaluate estrogen’s role in the neurovascular unit, simplistically comprised of the neurons, glia, and endothelial cells, as well as the processes of inflammation, and hemostasis/thrombosis. Besides the role of estrogen there are many gaps of knowledge about issues specific to women and stroke.

Summary of Review—A multidisciplinary workshop was held in August 2005 to summarize the current evidence for estrogen and, more generally, stroke in women, and to provide recommendations for future basic, preclinical, and clinical research studies.

Conclusions—These studies may ultimately change the approach to stroke prevention and treatment in women. (Stroke. 2006;37:2387-2399.)

Key Words: estrogen ▪ minority groups ▪ stroke ▪ women

Stroke is the third leading cause of death in men and women in the US, but there are some important sex differences that are still poorly understood. Some of these differences may be related to the onset of stroke at older ages in women than men. However, even after adjustment for age, women have poorer outcomes, such as greater pre- and poststroke disability, a higher likelihood for admission to nursing facilities, and greater mental impairments than men.1,2 Stroke risk factors unique to women include pregnancy,3 oral contraceptive use,4,5 and the use of exogenous hormonal treatment for menopausal symptoms.

Men and women may respond differently to stroke prevention strategies, as shown in 2 different gender-specific cohorts. In the Women’s Health Study, women aged 45 years or older and at low risk for cardiovascular disease had a reduced risk of stroke with low-dose aspirin,6 whereas men in the Physician’s Health Study showed no benefit from aspirin.7 However, because of the differences in timing of these 2 studies, large cohorts of women and men with the same level of stroke risk need to be treated with aspirin concurrently in order to confirm this apparent difference.

The accumulating evidence about gender differences in stroke treatment and outcomes, as well as the results of Women’s Health Initiative reporting an apparent stroke risk with hormone therapy,8,9 have raised many questions about stroke risk, treatment, and the role of estrogens. Therefore, the purpose of the conference “Advancing the Study of Stroke in Women” was to...
gather a multidisciplinary group of researchers to address the gaps in knowledge about issues specific to stroke risk in women with a focus on estrogen. Included were representatives from the fields of neurology, neurobiology, internal medicine, cardiology, hematology, pharmacology, vascular physiology, obstetrics/gynecology, and epidemiology. The concept of the neurovascular unit was used to help understand the cumulative effects of estrogen on stroke. It simplistically consists of neurons, glia and endothelium, and provides a conceptual method for evaluating stroke mechanisms.10 The following is a summary of the conference topics and recommendations for future research on stroke in women to address key gaps in knowledge.

Estrogen and Stroke in Women: Reviewing the Evidence

Although the use of hormone therapy was associated with a reduction in the risk of heart disease by about 50% in observational studies, the results for stroke have been far less clear (Table 1). With the exception of one that reported an increased risk,11 and one that reported a reduction in stroke risk with hormone therapy,12 the majority of studies have not found any effect.13–23 When analyzing subgroups of women with strokes, hormone therapy has had a differential effect on stroke risk depending on the presence of risk factors. For example, women with hypertension using hormone therapy had an increased risk of ischemic stroke compared with women without hypertension.24

Randomized controlled trials were implemented to determine whether hormone therapy was effective for secondary prevention of cardiovascular disease, including stroke (Table 2). Both the Heart and Estrogen/progestin Replacement Study (HERS) and the Women Estrogen Stroke Trial (WEST) reported no significant overall increase in risk for stroke with hormone therapy.25,26 However, the WEST also reported an increased risk of stroke in the first 6 months of treatment, a nearly 3-fold increased rate of fatal strokes (relative risk [RR] 2.9; 95% CI, 0.9 to 9.0), and worse neurological deficits in the estradiol group compared with placebo.26 Therefore, these studies showed that there was no preventive benefit of estrogen treatment for stroke prevention, and it may be harmful in high risk women with established vascular disease.

In the only randomized trial of postmenopausal hormone therapy in older postmenopausal women deemed to be low risk, the Women’s Health Initiative (WHI) showed a 40% increased risk of stroke (RR 1.41; 95% CI, 1.07 to 1.85) and 30% increased risk of first coronary heart disease events (RR 1.29; 95% CI, 1.02 to 1.63) with combined hormone therapy (Table 2).27 In addition, there was an increased risk of ischemic stroke in the group of women with hysterectomies randomized to conjugated estrogens alone (RR 1.39; 95% CI, 1.10 to 1.77).9 Although the number of events was higher in the hormone therapy groups from both arms of the WHI, the strokes were not more disabling.8 The absolute risk associated with hormone therapy was 8 strokes per 10 000 women per year of use.

Multiple subgroup analyses of women with hypertension, diabetes, or cigarette smoking were performed to determine whether these characteristics might identify them as being at high risk before initiation of hormone therapy. However, none of these characteristics appeared to place women at higher risk of stroke with hormone therapy.

### Table 1. Risk of Stroke From Observational Studies of HT

<table>
<thead>
<tr>
<th>Author</th>
<th>Cohort</th>
<th>HT Type</th>
<th>End Point</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lokkegaard</td>
<td>Danish Nurse Study</td>
<td>Estrogen</td>
<td>Ischemic stroke</td>
<td>0.94</td>
<td>0.40–2.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Estrogen with progestin</td>
<td>Ischemic stroke</td>
<td>1.99</td>
<td>1.09–3.64</td>
</tr>
<tr>
<td>Petitti</td>
<td>Northern CA Kaiser Permanent</td>
<td>Estrogen alone or with progestin</td>
<td>Ischemic stroke</td>
<td>1.03</td>
<td>0.65–1.65</td>
</tr>
<tr>
<td>Sourander</td>
<td>Turku, Finland, Mammography Study</td>
<td>Estrogen</td>
<td>Stroke</td>
<td>0.86</td>
<td>0.42–1.75</td>
</tr>
<tr>
<td>Pfeffer</td>
<td>California retirement community</td>
<td>n/a</td>
<td>Stroke</td>
<td>1.12</td>
<td>0.79–1.57</td>
</tr>
<tr>
<td>Pedersen</td>
<td>Danish National Patient Register</td>
<td>Estrogen</td>
<td>Ischemic stroke</td>
<td>1.24</td>
<td>0.91–1.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Estrogen with progestin</td>
<td>Ischemic stroke</td>
<td>1.27</td>
<td>1.00–1.62</td>
</tr>
<tr>
<td>Wilson</td>
<td>Framingham Heart Study</td>
<td>n/a</td>
<td>Ischemic stroke</td>
<td>2.60</td>
<td>n/a</td>
</tr>
<tr>
<td>Fung</td>
<td>Rancho Bernardo, CA</td>
<td>All estrogens</td>
<td>Nonfatal stroke and TIA</td>
<td>3.02</td>
<td>0.70–13.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All estrogens</td>
<td>Fatal stroke</td>
<td>0.92</td>
<td>0.34–2.49</td>
</tr>
<tr>
<td>Falkeborn</td>
<td>Uppsala, Sweden</td>
<td>All estrogens</td>
<td>Ischemic stroke</td>
<td>0.91</td>
<td>0.76–1.09</td>
</tr>
<tr>
<td>Rosenberg</td>
<td>Northern CA Kaiser Foundation</td>
<td>All estrogens</td>
<td>Stroke</td>
<td>1.16</td>
<td>0.75–1.77</td>
</tr>
<tr>
<td>Lemaitre</td>
<td>Seattle, WA, Group Health Cooperative</td>
<td>Estrogen</td>
<td>Ischemic stroke</td>
<td>0.97</td>
<td>0.69–1.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Estrogen with progestin</td>
<td>Ischemic stroke</td>
<td>0.94</td>
<td>0.72–1.23</td>
</tr>
<tr>
<td>Grodstein</td>
<td>Nurses’ Health Study</td>
<td>Conjugated estrogen =0.625 mg</td>
<td>Stroke</td>
<td>1.35</td>
<td>1.08–1.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Estrogen with progestin</td>
<td>Stroke</td>
<td>1.45</td>
<td>1.10–1.92</td>
</tr>
<tr>
<td>Finucane</td>
<td>National Health and Nutrition Examination Survey</td>
<td>n/a</td>
<td>Stroke</td>
<td>0.69</td>
<td>0.47–1.00</td>
</tr>
<tr>
<td>Lindenstrom</td>
<td>Copenhagen City Heart Study</td>
<td>n/a</td>
<td>Stroke HRT and Smoking</td>
<td>0.57</td>
<td>0.29–1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HRT and No smoking</td>
<td>1.01</td>
<td>0.55–1.84</td>
</tr>
<tr>
<td>Angeja</td>
<td>National Registry of Myocardial Infarction-3</td>
<td>n/a</td>
<td>Ischemic stroke</td>
<td>0.89</td>
<td>0.66–1.18</td>
</tr>
</tbody>
</table>

HT indicates hormone therapy; TIA, transient ischemic attack; n/a, not applicable.
Some of the criticisms of the WHI design was that it included women older than would normally be prescribed hormone therapy for menopausal symptoms, that women with risk factors should not have been considered “healthy” (in the absence of quantitative measures of vascular health such as carotid intimal thickening), and that continuous rather than cyclic medroxyprogesterone was used in the combined hormone therapy arm of the study.\(^2^8\) To address these concerns, the Kronos Early Estrogen Prevention Study (KEEPS) is an ongoing prospective, randomized, placebo-controlled study of oral compared with transdermal estrogens with pulsed progesterin in women early after menopause. The primary outcomes will be vascular disease measured by progression of carotid intimal medial thickness and coronary artery calcification.\(^2^9\) The KEEPS cohort is important because it matches the characteristics of women who might benefit from postmenopausal hormone treatment, as well as studying formulations of hormones that might reduce risk.

### Research Gaps

The characteristics that might identify women at higher risk of stroke with hormone therapy use are still unknown.

### New Ways to Think About Estrogen and Injury in the Central Nervous System

The potential impact of estrogens on stroke has been studied extensively over the last decade. The interest has arisen in part because of the hormone’s putative role in protecting women from cardiovascular disease and stroke. Furthermore, preclinical studies using animal or cell models have consistently shown that treatment with estrogens or estrogen mimetics reduces tissue damage from ischemia and toxicity. For example, XY neurons are more susceptible to glutamate or peroxynitrite exposure than are XX cells, whereas XX cells are more sensitive to proapoptotic stimuli.\(^3^4\) Cell death after oxygen-glucose deprivation is decreased in female versus male primary astrocyte cultures\(^3^5\) and in female-derived hippocampal slices.\(^3^6\) Such experiments conducted in steroid-free media suggest that some mechanisms of injury (or survival) may be in part linked to the genetic sex of the cell. Consistent with this concept, new studies confirm that molecular death pathways are sex-specific; and 3) pharmaceutical agents under investigation for stroke do not necessarily benefit both females and males.

When female and male animals are compared, a male phenotype of “ischemic sensitivity” is found, even in the presence of comorbidities such as diabetes and hypertension.\(^3^3\) More recently, in vitro experiments using “sex-ed” cultures demonstrated that XX versus XY cells respond differently to simulated ischemia and toxicity. For example, XY neurons are more susceptible to glutamate or peroxynitrite exposure than are XX cells, whereas XX cells are more sensitive to proapoptotic stimuli.\(^3^4\) Cell death after oxygen-glucose deprivation is decreased in female versus male primary astrocyte cultures\(^3^5\) and in female-derived hippocampal slices.\(^3^6\) Such experiments conducted in steroid-free media suggest that some mechanisms of injury (or survival) may be in part linked to the genetic sex of the cell. Consistent with this concept, new studies confirm that molecular mechanisms of ischemic cell death, eg, inducible and neuronal nitric oxide synthase and poly(ADP-ribose) polymerase, is only beneficial in the males.\(^3^7,3^8\) A logical extension of this concept is that preclinical data should be obtained in both sexes to avoid erroneous conclusions, just as clinical trials of new therapies and drugs require representation of both sexes. For example, new data with the selective \(\kappa\) opioid receptor agonist BRL 52537 hydrochloride show that the drug could be promising because of its long therapeutic window and low toxicity. Notably, it is effective only in male animals.\(^3^9\) Accordingly, further testing in human stroke will require careful stratification by sex if BRL’s therapeutic benefits are to be fully evaluated.

Another new way of considering estrogen effects is to emphasize its efficacy as a treatment that minimizes brain injury when stroke does occur. We reviewed literature over the last 10 years and assembled an informal scorecard for estrogen. Exogenous treatment was reported to reduce histological damage or behavioral deficit in over 50 studies of rodent global or focal cerebral ischemia (versus 4 studies that failed to show benefit) and in 22 in vitro studies of neurons

### Table 2. Risk of Stroke From Randomized Controlled Trials of HT

<table>
<thead>
<tr>
<th>Author</th>
<th>Cohort</th>
<th>HT Type</th>
<th>End Point</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simon(^2^5)</td>
<td>HERS</td>
<td>CEE/MPA</td>
<td>Ischemic</td>
<td>1.18</td>
<td>0.83–1.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fatal</td>
<td>1.61</td>
<td>0.73–3.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nonfatal</td>
<td>1.18</td>
<td>0.83–1.66</td>
</tr>
<tr>
<td>Viscoli(^2^6)</td>
<td>WEST</td>
<td>17(\beta)-Estradiol</td>
<td>Stroke or death</td>
<td>1.10</td>
<td>0.80–1.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nonfatal</td>
<td>1.00</td>
<td>0.70–1.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ischemic</td>
<td>1.00</td>
<td>0.60–1.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fatal</td>
<td>2.90</td>
<td>0.90–9.00</td>
</tr>
<tr>
<td>Wassertheil-Smoller(^d)</td>
<td>WHI</td>
<td>CEE/MPA</td>
<td>Ischemic</td>
<td>1.44</td>
<td>1.09–1.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fatal</td>
<td>1.20</td>
<td>0.58–2.50</td>
</tr>
<tr>
<td>WHI Writing Group(^d)</td>
<td>WHI</td>
<td>CEE</td>
<td>Stroke</td>
<td>1.39</td>
<td>1.10–1.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nonfatal</td>
<td>1.39</td>
<td>1.05–1.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fatal</td>
<td>1.13</td>
<td>0.54–2.34</td>
</tr>
</tbody>
</table>

HT indicates hormone therapy; CEE, conjugated equine estrogen; MPA, medroxyprogesterone acetate.
(versus 0 that failed to show benefit). A large variety of naturally occurring estrogens and nonestrogen receptor–binding estradiol analogues have all been shown to be effective.40 This scorecard suggests that estrogens have been one of the most widely studied neuroprotective agents in recent times. Yet none of the estrogens have been evaluated in humans for treatment of stroke, despite evidence that even single steroid injection can reduce ischemic damage. It is therefore of interest to consider estrogen as a protective prototype molecule and evaluate how this molecule can be adapted to treat neuropathology in both women and men.

Research Gaps
The effect of estrogen administration as a neuroprotectant in acute stroke has not been evaluated.

Estrogen and Endothelial Function
Experimental evidence indicates that estrogen modulates production of endothelium-derived factors including nitric oxide, angiotensin-converting enzyme, cyclooxygenase metabolites of arachidonic acid (prostacyclin, thromboxane), endothelin-I, endothelin-derived hyperpolarizing factor, natriuretic peptides, superoxide radicals and tissue factor pathway inhibitor.41 These varied multiple actions of estrogen on the endothelium may, in part, explain sex-differences in the efficacy of cardiovascular prophylactic therapies such as aspirin and angiotensin-converting enzyme inhibitors.6 This explanation is plausible because the modulation of the production of endothelium-derived growth factor by estrogen favors those factors which are vasodilatory and antiinflammatory.42

Effects of estrogen on the endothelium are mediated by ligation of one or both of 2 identified receptors (estrogen receptor α, ERS1; estrogen receptor β, ERS2).43 Ligation of estrogen receptors located in the membrane caveolae stimulate intracellular signaling pathways including activation of membrane ion channels, changes in intracellular calcium, AKT, mitogen activated (MAP)-kinase and activation of nitric oxide synthase. These changes occur within minutes and because they are observed in the absence of gene transcription are classified as nongenomic effects.44,45,46 However, in addition to these rapid changes in the intracellular milieu, ligand-bound receptors affect gene transcription.47 Therefore, these combined effects of sex-steroids affect threshold, gain and duration of cell signaling, not only in the endothelium, but also in each compartment of the neurovascular unit.

Because most cellular effects of estrogen are mediated by receptors, genetic variation in estrogen receptors might be associated with specific vascular anomalies. Indeed, polymorphisms in estrogen receptors have been associated with increased incidence of cardiovascular disease and hypertension in men and women.48,49,50 As the estrogen receptors also bind metabolites of estrogen,51 genetic variation in enzymes which metabolize estrogen within various tissues will affect an individual’s response to an estrogen compound or contribute to defining an “at risk” endothelial phenotype including expression of inflammatory surface adhesion molecules.52,53,54,55

In addition to sustaining a vasodilatory, antiproliferative and antiadhesive endothelium, estrogen may facilitate regeneration of endothelium through increases in circulating endothelial progenitor cells.56 The degree to which changes in these circulating pleiotropic cells contribute to cardiovascular disease and the influence of sex steroids on these processes is an exciting new area of research.56,57

Research Gaps
How changes in hormonal status during the transition to menopause affect endothelial function in general, and in the cerebral circulation in particular, are poorly understood. New research should focus on the cellular and molecular pathways by which estrogen and other steroid hormonal treatments regulate cellular metabolism and oxidative stress via modulation of mitochondrial functions.

The Neurovascular Unit
A growing body of data indicates an inter-relationship between vascular and neuronal injury, particularly during focal cerebral ischemia. In normal cerebral tissue, control of vascular tone is accomplished via neurovascular coupling.58 Together, these suggest that events which affect neurons and their supporting cells are likely to have direct consequences to the regional microvascular bed and the possibility that communication may occur in the afferent direction as well.

A conceptual framework for addressing some of these unknowns is provided by considering local neurovascular and neural interactions as a unit.59,60,61 Cerebral microvessels consist of endothelial cells closely apposed to astrocyte end-feet across the basal lamina matrix.59 Both cell types cooperate to form the endothelial cell blood–brain barrier and the intervening basal lamina.62,63,64,65,66 Astrocytes also provide communications and support local neurons.87,68 Hence, the “neurovascular unit” may be typified as the complex of microvessels (of which endothelium-matrix-astrocyte end-foot complex is an integral part), their astrocyte projections, neurons served by their projections, and axons. Supporting cells, including pericytes, microglia, and oligodendrocytes may affect the functions of the unit (Figure).

Focal ischemia disturbs these relationships at a number of levels. In addition to direct effects on astrocyte and neuronal function, 1) microvascular permeability exposes glia and neurons to toxic plasma products, 2) the (extracellular) matrix within microvessels and surrounding cells of the “neurovascular unit” is rapidly degraded by proteases generated within the “unit,” and 3) immune-competent cells generate cytokines, chemokines, and other products. These affect the function of cells within the “unit,” can serve processes that extend cellular injury, and together with subsequent cellular inflammation, lead to infarction and destruction of the neurovascular unit. In this setting, endothelial cells are more resistant than extravascular cells.

This conceptualization provides broad opportunities for experimental tests. It allows not only for the examination of the individual cell and structural components in culture, but also exploration of the function of the unit in more complex settings, including animal models. Aspects of this concept which are not known (but can stimulate future research) are: 1) how does alteration of astrocyte function affect endothelial cell and neuron responses to stimulation (eg, during ischemia)?; 2) how does
selective alteration of the basal lamina matrix affect neuron function?; 3) how does axonal injury alter local microvascular flow?; and 4) can luminal events be transmitted via endothelial cell activation to neuronal response? In a broader context 1) how does the “neurovascular unit” differ in various locations within the cerebral hemisphere?, 2) do “units” in cognate brain tissues of different species differ, and in what respects?, and 3) do sex differences exist in the “neurovascular unit?” This latter query is very relevant to the thrust of the current discussion. One recent study has indicated different numbers of astrocytes and microglia in female compared with male animals. Intriguingly, there is evidence that isolated astrocytes derived from male mice behave differently to ischemia compared with those from female mice. This raises the possibility that the sex and source of other components of the neurovascular unit, or the entire unit itself, could define the responses to ischemic injury, dementing disorders, neuronal degenerating disorders, or local inflammation.

**Research Gaps**

Sex-specific differences in the neurovascular unit and the response to ischemia have not been adequately explored. Those differences may be more or less apparent in individual cells of the “unit” (eg, endothelial cells, astrocytes, neurons) or in the function of the “unit” overall.

**Estrogen and Glial Function**

Astrocytes and microglia, 2 prominent forms of glia, are important cellular components of the neurovascular unit and contribute to the development and maintenance of tight junction proteins, blood–brain barrier permeability, and participate in an immune response when challenged by injury or infection. Under normal conditions, the local neurovascular environment maintains the permeability of the barrier within physiological limits. Changes in this environment induced by either cardiovascular events such as thrombosis or by central nervous system events such as brain injury alter the permeability of the tight junctions and initiate an immune response. Astrocytes and microglia in the neurovascular compartment participate in this response by releasing cytoactive factors that synergistically act to further promote inflammation. The subsequent inflammatory cascade results in severe changes to blood–brain barrier permeability, to infiltration of leukocytes into the brain and extends the inflammatory response to nearby brain parenchymal cells.

Abatement of inflammation in any tissue including the brain depends on receptor-specific signaling, the production of anti-inflammatory agents or the presence of global factors that alter the set point for inflammation. Anti-inflammatory cytokines such as interleukin (IL)-10, IL-4 and IL-13 are
induced by the same signals that initiate the proinflammatory cascade, and when released into the environment, they counteract production and activities of proinflammatory cytokines. Finally, the set point of inflammation can be altered by “global” factors; that is, those factors that may be part of the basic make-up of the individual.

Estrogen has been shown to affect macrophage function and the immune response based on various experimental approaches. For example, exposure of immune cells in culture to physiological levels of estrogen reduces the production of proinflammatory cytokines, reactive nitrogen species and other factors involved in classic activation of the innate immune response. Similar results have now been demonstrated in a wide number of cell models including primary brain microglia or peritoneal macrophages from rat and mouse, primary human macrophages isolated from blood and macrophage cell lines from all species. Astrocyte activation is also downregulated by the exogenous addition of 17-estradiol in culture. Although contradictory data exist, in general estrogen suppresses inflammation.

Specific gene polymorphisms, such as the presence of the APOE4 gene allele, alter the responsiveness of immune cells to estrogen. APOE is the gene that encodes a lipid transport protein known as apolipoprotein E (apoE). In addition to serving to carry cholesterol and lipids to cells in the brain, apoE regulates immune function. Importantly, the APOE gene has polymorphisms that allow the gene to be commonly expressed as 3 different isoforms, APOE2, APOE3 and APOE4. Each of the genes codes for a slightly different apoE protein (APOE2, APOE3, APOE4). These isoforms are best known for their relationship to Alzheimer disease such that carriers of an APOE4 gene allele have an increased risk for Alzheimer disease. Although the mechanism of this effect is not yet known, the 14% of the human female population that express APOE4, the normal gene allele found in the majority of people. The possibility exists that other genes can be identified that are associated with both estrogen-mediated events and inflammation. Searching for these critical genetic factors may help us to understand the variability in a woman’s response to estrogen.

Research Gaps

Further research is necessary to determine which genetic factors can override estrogen’s beneficial actions related to inflammation and stroke risk.

Potential Role of Inflammatory Biomarkers of Risk in Women

Inflammation-related markers (Table 3) are associated with the risk of incident and recurrent cardiac events, and probably stroke. Acute phase proteins, particularly C-reactive protein (CRP), have been most extensively studied. CRP may be a marker of the inflammation that is present in atherosclerosis, or alternatively, play a direct role in atherosclerosis.

CRP has been associated with incident cardiovascular events including stroke in populations of women. In the Women’s Health Study, high sensitivity- (hs) CRP, IL-6, soluble intercellular adhesion molecule-1 and serum amyloid A were all associated with incident cardiovascular events, including stroke. However, hs-CRP was the only inflammatory marker that was independently associated with risk, and the inclusion of hs-CRP improved the predictive ability of the models over those containing lipid values and other risk factors alone (P<0.001), both in women and in men. In the Women’s Health Initiative, hs-CRP was not independently associated with future cardiovascular events after both leukocyte count and hs-CRP were included in the model, although leukocyte count maintained its association.

The relationship of CRP to incident stroke risk may depend on the study design and population studied. Many studies focused on predominantly healthy middle-aged individuals without a significant burden of risk factors. Hs-CRP levels in the highest quartile were associated with a doubling of risk of stroke among men in the Physicians’ Health Study and in the Framingham Heart Study (FHS), whereas Framingham women in the highest quartile tripled their risk. Among women in the Framingham cohort, but not men, moreover, the increased risk was present even after adjusting for confounders. The greatest predictive value of hs-CRP may thus be found among those with low baseline risk.

CRP is increased in women compared with men, and in postmenopausal women using hormone replacement therapy. Whether the hormone-associated increase in hs-CRP levels translates into an increased risk of stroke remains uncertain. There is also evidence that transdermal estrogen preparations have less of an effect on hs-CRP levels than oral preparations.
Phytoestrogens also do not appear to raise hs-CRP levels.\textsuperscript{94} Because the effect of hormone therapy on inflammatory markers is not uniform for all markers, and many markers, including tumor necrosis factor-\textit{\alpha} and cellular adhesion molecules, actually decrease with hormone therapy use, it remains likely that the increase in hs-CRP does not represent a generalized increase in systemic inflammation.\textsuperscript{95}

Despite the possible proinflammatory effects of hormone therapy noted in clinical studies, estrogen has been shown to have anti-inflammatory actions in premenopausal women, with a shift toward proinflammatory cytokine production through the menopausal transition.\textsuperscript{96} Therefore, the responses of these cytokines and other inflammatory markers in the presence of estrogen depends on whether they have been tested in human, animal, or cell culture models, and which type of estrogen was studied.\textsuperscript{96} Studies performed in women differ depending on whether the population is premenopausal or postmenopausal.\textsuperscript{96} In addition, there are genetic polymorphisms that may significantly alter this response, as shown with \textit{APOE} polymorphisms.\textsuperscript{77}

\textbf{Research Gaps}

The optimal markers for determining risk of stroke in women at low or high risk and in understudied populations are not known. In addition, the role of various therapies that have been shown to lower CRP (ie, statins) in women at risk or who are using hormone therapy have not been studied.

\textbf{Estrogen, Hemostasis, and Genetic Predisposition to Thrombosis}

Estrogen’s influence on hemostasis is recognized primarily from its association with an increased risk of venous thromboembolism (VTE). Overall, hormone therapy (estrogen alone or in combination with progestin) increased the risk of VTE by 2- to 6-fold in various randomized and nonrandomized studies.\textsuperscript{97,98,99,100,101} Estrogen has a variable affect on many different markers of thrombosis activation. Although the estrogen-mediated decrease in plaminogen activator inhibitor-1 (PAI-1) is predicted to be antithrombotic, the majority of hemostatic biomarkers change in a prothrombotic direction, with increases in factor VII activity, D-dimer and prothrombin F1.2, and decreases in anti-thrombin III, tissue factor pathway inhibitor and tissue plasminogen activator.\textsuperscript{102,103,104,105} Despite all of these data, the precise mechanism of an estrogen-associated increase in risk for venous (as well as arterial) thrombotic events remains unknown. Prothrombic genetic variants may identify a susceptible cohort of women at greater risk of thrombosis in the setting of estrogen therapy.

\textbf{Factor V Leiden and Prothrombin 20210A}

Two common mutations that increase the risk for VTE are factor V Leiden (FVL) and 20210A. Significant increases in VTE risk were reported in women who were carriers of FVL and taking hormone therapy compared with non-FVL women taking placebo (\(P = 0.0015\)) from the HERS and the Estrogen Replacement and Atherosclerosis (ERA) trials.\textsuperscript{106} Similarly, Psaty et al reported an 11-fold increase in MI risk in current hormone therapy users with hypertension who had the prothrombin variant compared with non–hormone therapy wild-type subjects.\textsuperscript{107} However, neither mutation has been examined in association with hormone therapy use and ischemic stroke. One potential explanation for ischemic stroke in hormone therapy users with either of these polymorphisms may lie in the potential for paradoxical embolism in the setting of a patent foramen ovale.\textsuperscript{108}

\textbf{PAI-1 (4G/5G)}

PAI-1 levels differ based on sex, menopause status, and the use of hormone therapy.\textsuperscript{109} This modulation of fibrinolysis by estrogen may be related in part to the 4G allele, which is associated with higher circulating levels of PAI-1 in vivo.\textsuperscript{110,111,112} Elevated PAI-1 levels, in turn, are associated with increased risk for VTE,\textsuperscript{111} MI,\textsuperscript{113,114} and stroke\textsuperscript{115} presumably by inhibiting the fibrinolytic cascade. One study of postmenopausal women with coronary artery disease showed that hormone therapy users had significantly lower PAI-1 levels than nonusers, but that this response was dependent on the presence of a 4G allele.\textsuperscript{116} Therefore, hormone therapy may modulate PAI-1 levels that may be beneficial in an otherwise higher-risk phenotype.

\textbf{\textit{PlA1/A2}}

The \textit{PlA2} allele is associated with increased platelet aggregability.\textsuperscript{117} In a meta-analysis of \textit{PlA1/A2} and risk of MI, the risk of the A2 allele was greatest among women; however, the confidence intervals were wide and included unity because of the relatively few data currently available in women.\textsuperscript{118} A separate study found no association between \textit{PlA2} and ischemic stroke in young women; however, subgroup analyses by race suggested that white women may be at higher risk with this polymorphism than black women.\textsuperscript{119} These data suggest that gender, race-ethnicity, and estrogen status may have a significant impact on the relationship between \textit{PlA1/A2} genotypes and risk for thrombosis.

\textbf{Research Gaps}

Clinical studies are needed to determine whether there are subgroups of women who could be stratified as high or low risk for thrombotic complications with hormone therapy use based on the presence of specific genetic polymorphisms.

\textbf{Analysis of High(er) Risk Populations}

\textbf{Stroke in Pregnancy}

The occurrence of stroke during pregnancy is infrequent, but it represents a potentially devastating event for a young woman who is starting a family. A recent analysis of the Nationwide Inpatient Sample revealed an all-stroke incidence of 34.2/100 000 deliveries.\textsuperscript{3} In a population-based study, the ischemic stroke rate was 11/100 000 deliveries, with the postpartum period posing the highest risk (RR 8.7).\textsuperscript{120} In this same cohort, intracerebral hemorrhage occurred at a rate of 9/100 000 deliveries, and similar to ischemic stroke, the highest risk was in the postpartum period (RR 28).\textsuperscript{120} Risk factors for pregnancy-associated stroke and transient ischemic attack include age >35 years, black ethnicity, hypertension, heart disease, smoking, diabetes, lupus, sickle cell disease, migraine headaches, alcohol and substance abuse, cesarean delivery, fluid, electrolyte and
Acid-base disorders, thrombophilia, multiple gestation, greater parity, postpartum infection, pre-eclampsia, and eclampsia. 3,121 There are multiple conditions that have been associated with both ischemic and hemorrhagic stroke in pregnancy and during the postpartum period. This review focused on pre-eclampsia/eclampsia, peripartum angiopathy, 122 and cerebral venous sinus thrombosis.

The proportion of patients with pregnancy-associated stroke who have pre-eclampsia or eclampsia is 25% to 45%. 130,132 A family history of pre-eclampsia conveys a relative risk of 2.9 for pre-eclampsia. 124 Having 2 or more first-degree relatives with cardiovascular risk factors is associated with double the risk of pre-eclampsia (RR 1.9), and having 2 or more first-degree relatives with heart disease or stroke is associated with a 3-fold risk (RR 3.2). 125 These data suggest that there may be genetic factors common to the risk of both pre-eclampsia and atherosclerotic disease.

The pathophysiology of pre-eclampsia involves endothelial dysfunction, which suggests there are parallels between pre-eclampsia and atherosclerosis. The first pathophysiological stage of pre-eclampsia consists of shallow cytotrophoblast invasion of maternal spiral arteries, resulting in hypoxic ischemic placental insufficiency. 126 The second stage results in the release by the hypoxic placenta of soluble factors into the maternal circulation that induce systemic endothelial dysfunction.

Angiogenic factors, such as vascular endothelial growth factor (VEGF) and secreted Flt-1 (s-Flt-1), may be the most important mediators of pre-eclampsia. 126 VEGF promotes vasodilation by increasing nitric oxide and prostacyclin production. Secreted Flt-1 (sFlt-1) antagonizes the activity of both VEGF and placental growth factor. It has been proposed that excess Flt-1 made by pre-eclamptic placentas may be responsible for pre-eclampsia by inducing a deficiency of VEGF and placental growth factor. 126

Based on available data, several hypotheses that link pre-eclampsia and stroke etiologies can be proposed: 1) pre-eclampsia/eclampsia-associated ischemic stroke is a complex disorder mediated in part by a genetically determined endolymphatic and thrombophilia; 2) pre-eclampsia/eclampsia-associated hemorrhagic stroke is additionally associated with a severe disturbance of cerebral autoregulation that may be genetically mediated; 3) postpartum angiopathy is a variant of pre-eclampsia/eclampsia in which a disturbance in cerebral autoregulation dominates the clinical presentation; 4) the changes in vascular biology associated with pre-eclampsia and gestational hypertension may account for the observed increased risk for ischemic stroke after the childbearing years. 127-129

Approximately 2% of pregnancy-associated strokes are attributable to venous thrombosis. 3 Although the puerperium and oral contraceptive use are commonly associated with cerebral venous sinus thrombosis (CVST), thrombophilic disorders are also important causes. 130 The case fatality rate of pregnancy-associated CVST has been reported to range between 4% to 36%. 131 The most frequent cause of death in one analysis of patients with CVST (n=27) was transtentorial herniation. The authors speculated that decompressive craniectomy may be indicated in the deteriorating patient. 131 The use of intramuscular urokinase for the treatment of pregnancy-associated CVST has also been reported. 132

Research Gaps
Factors that predispose a woman to develop risk factors for pregnancy-associated stroke such as pre-eclampsia/eclampsia or CVST are incompletely understood.

Stroke in Blacks: Women and Research Gaps
Epidemiological Trends
Stroke mortality has traditionally been high in the black community, and black women and men are at higher risk of stroke death compared with most other sex and race-ethnic groups in the US. 133 Risk of incident stroke is also high among black women and men, and black women may have the highest prevalence of stroke. 134,135,136,137,138 These stroke rate disparities are most pronounced in young and middle-aged blacks and in the Stroke Belt, an area located in the Southeast US where both blacks and whites have high stroke risk. However, stroke is not uniform in the Stroke Belt, but may show variation in rates in contiguous areas. 139 Understanding why black women and men have such high stroke risk and mortality and why there is geographic variation merits further study to help us better understand this disparity in health outcomes. 140

Stroke Subtype and Risk Factor Burden
The frequency of stroke subtype and cardiovascular risk factor burden differs by race-ethnic group, which may help explain why stroke risk is so high in blacks. For example, blacks have a higher risk of lacunar infarction but a lower risk of cardioembolic stroke, 141,142,143 a finding that may indicate a different cardiovascular risk profile or a different influence of these risk factors in this group. Hypertension, the most important modifiable risk factor for stroke, may develop earlier in life, be more severe and more prevalent, and be less successfully controlled in blacks than many other race-ethnic groups. 144,145

A significant proportion of hypertensive blacks are women of child bearing potential, which raises the issue of birth control. Although oral contraceptive therapy is simple to use, reliable, and relatively inexpensive, its use in hypertensive black women warrants caution, because it has been shown that, just as with menopausal hormone replacement therapy, prolonged exposure to low-dose oral contraceptives in this and other high risk populations may increase the risk of stroke.146

Also, the frequency of obesity, diabetes mellitus, and cigarette smoking is generally higher in blacks than many other race-ethnic groups in the US. 147 Furthermore, the metabolic syndrome, which represents a clustering of cardiovascular risk factors, is highly prevalent in the black community. 148 Black women have a 57% higher rate of metabolic syndrome compared with black men. Hormonal and genetic pathways by which stroke and cardiovascular disease may occur need to be better elucidated. 149,150,151,152

Clinical Trials
Optimal prevention and management of stroke in blacks requires careful study with adequate enrollment of participants within this high-risk population. Studies involving large numbers of blacks are generally lacking, limiting our ability to make decisions regarding causes and treatment of stroke in this group. 153 With the exception of the African American Antipla-
with 100% black participation, there is relatively little data in the stroke clinical trial literature to help us definitively understand prevention and treatment of stroke among blacks. Of the recent hormone therapy prevention trials, for example, only the WEST trial reached a participation rate similar to the proportion of blacks in the US population. Reasons for inadequate participation are multifactorial and reside within many levels of the healthcare system as described previously by the AAASPS investigators and the Institute of Medicine. Although some reasons are well delineated (i.e., mistrust), others remain unclear and are in need of further investigation.

Research Gaps
Understanding of the reasons for race-ethnic differences in stroke incidence and mortality are incomplete, and including large numbers of minority subjects in clinical trials needs to be pursued.

Workshop Recommendations
By recognizing gaps in our knowledge of stroke in women with an emphasis on estrogen, the workshop scientists from a variety of disciplines recommended studies that will address these gaps. The workshop recommendations reflect new research that needs to be designed from the perspectives of both basic science and clinical research.

Summary Statements: Basic Science Research
1. Define the responses of the neurovascular unit and its individual components to ischemic injury in the context of sex origin.
2. Develop consistent animal models of cardiovascular changes that translate into a stroke risk profile.
3. Define which stimuli precipitate stroke events, as well as the influence of sex steroids on these processes.
4. Delineate the mechanism by which sex steroids and their mimetics act as neurovascular protectants in both stroke and neurodegenerative disease models.
5. Design and perform additional studies that will delineate the actions of selective estrogen receptor modulators in experimental animal models of stroke.
6. Design and perform studies that will delineate the mechanism of hormone (including estrogen, progesterone and selective estrogen receptor modulators) effects on endothelial cells, thrombosis markers, megakaryocytes and platelets.

Summary Statements: Clinical Research Initiatives
1. More prospective controlled studies are necessary to understand and evaluate the incidence of stroke and how to mitigate stroke risk, in women using hormone therapy that is initiated specifically for treatment of menopausal symptoms.
2. Research is needed that is focused on sex differences in the perception of risk, diagnosis, treatment, and prevention for stroke compared with, and independent of, heart disease.
3. There is a need for better education regarding stroke symptoms, risk factors and available therapies and interventions (acute and preventive) for women and their care providers.

4. The characteristics of women who may be candidates for hormone therapy and considered to be at high risk for cardiovascular disease need to be better defined. Similarly, whether the initiation of aspirin therapy at the same time as oral or transdermal hormone therapy in these women might provide protection against adverse cardiovascular events needs to be assessed.
5. Further research should be focused on the impact of withdrawal of estrogen/progesterin therapy on stroke risk.
6. Determine whether and how other formulations of hormone therapy, such as transvaginal versus transdermal, reduce or increase stroke risk. The influence of these formulations of hormone on thrombosis and inflammation also needs to be delineated.
7. Future research regarding inflammatory markers and stroke risk in women should focus on: 1) identifying optimal markers; 2) use of existing epidemiological studies and clinical trials with stored blood specimens to efficiently study whether these markers predict risk of first stroke among postmenopausal women and recurrent stroke among stroke survivors; 3) whether changes in inflammatory cytokines are a cause or effect of stroke risk; 4) differences in women of different race-ethnic groups, including understudied populations such as blacks, Hispanics, Asians, and others; 5) role of statins and other therapies in reducing levels of these markers and modifying the risk associated with them. These recommendations are consistent with those made by the CDC/AHA consensus conference.

Research Gaps in High(er) Risk Populations

Stroke in Pregnancy
The genetic link between pre-eclampsia, endothelial dysfunction, and stroke or cerebrovascular complications during pregnancy requires further exploration. The design of studies to better understand stroke in pregnancy and pre-eclampsia could involve patients with pre-eclampsia, pre-eclampsia and stroke, and eclampsia with or without stroke. Candidate genes should be investigated with control subjects matched by age, race/ethnicity, and parity. In this young case cohort, genetically informative parents and siblings should be frequently available, thereby making use of the transmission disequilibrium test and its variants feasible.

Stroke in Black Women
The following stroke-related areas are in need of further research to help us better understand disparities for stroke in black women:

1. Reasons for excess stroke risk and mortality including identification of hormonal (oral contraception and hormonal therapy) and genetic mechanisms for excess burden of stroke and cardiovascular disease.
2. Reasons for geographic variations in stroke risk.
4. Reasons for lower participation rates of blacks in stroke clinical trials so that future trials will have greater participation of high risk groups.
5. Implementation of clinical trials suitably powered to determine the efficacy of stroke therapies.
Conclusions

The research gaps that were summarized and identified by the attendees of the conference have implications for preventing and treating stroke in women at all stages of the life cycle. Thus, the study of stroke in women requires multidisciplinary collaborations of basic scientists, neurologists and obstetricians as well as other subspecialists represented at this conference. In general, the workshop members discussed ways to achieve a better understanding of the phenotypic “fingerprint” of women at risk. This would include analyses of clinical characteristics and outcomes, imaging results, biomarkers (DNA, RNA, proteins), and tissue banking. This effort also requires the collaboration of investigators of existing clinical trials and registries to establish common data elements for further data mining. In addition, research is necessary to re-examine the inclusion/exclusion criteria for studies of hormone therapy, the mode and duration of hormone therapy, the timing of additional vascular interventions, and the use of measures of disease progression, such as carotid intimal medial thickness, endothelial function, global tests of coagulation and other novel humoral biomarkers.

Appendix

ASSW Conference Organizers: Cheryl Bushnell, MD, MHS; Patricia Hurn, PhD; and Carol Colton, PhD.

ASSW Working Group members: Virginia M. Miller, PhD; Gregory del Zoppo, MD; Mitchell S.V. Elkind, MD; Barney Stern, MD; David Herrinton, MD; GWendolyn Ford-Lynch, MD; Philip Gorelick, MD, MPH; Andra James, MD, MPH; Candice Brown, PhD; Emily Choi, MD; Paul Bray, MD; L. Kristin Newby, MD, MHS; Larry Goldstein, MD; James Simpkins, PhD; Phyllis Wise, PhD; Louise McCullough, MD, PhD; Ralph Sacco, MD, MS; Margaret Pykett, MD; Robinrey, MD; Virginia Howard, MS; George Howard, PhD; Richard Becker, MD; Pascal Goldschmidt, MD; Daniel Laskowitz, MD, MHS; Donald McDonnell, PhD; Kenneth Korach, PhD; Gretchjen Tietjen, MD; Thomas Ortel, MD, PhD; Louis Caplan, MD.

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Disclosures

None.

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Cheryl D. Bushnell, Patricia Hurn, Carol Colton, Virginia M. Miller, Gregory del Zoppo, Mitchell S.V. Elkind, Barney Stern, David Herrington, Gwendolyn Ford-Lynch, Philip Gorelick, Andra James, Candice M. Brown, Emily Choi, Paul Bray, L. Kristin Newby, Larry B. Goldstein and James Simpkins

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