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Sex Differences in Stroke: The Contribution of Coagulation

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Abstract

Stroke is now the leading cause of adult disability in the United States. Women are disproportionately affected by stroke. Women increasingly outnumber men in the elderly population, the period of highest risk for stroke. However, there is also a growing recognition that fundamental sex differences are present that contribute to differential ischemic sensitivity. In addition, gonadal hormone exposure can impact coagulation and fibrinolysis, key factors in the initiation of thrombosis. In this review we will discuss sex differences in stroke, with a focus on platelets, vascular reactivity and coagulation.

Keywords

Cerebral Ischemia; thrombosis; estrogen; women; vasculature

Introduction

Stroke

Each year, 15 million people worldwide are affected by stroke, resulting in 5 million deaths and 5 million cases of long-term disability. In the United States, stroke is the fourth most common cause of death and the leading cause of disability.¹ The American Heart Association predicts that by 2030, the direct cost of caring for stroke patients will triple from \$61.55 billion to \$183.13 billion, due in large part to increasing life expectancy and expansion of the US aging population; after 55 years of age, the risk of stroke more than doubles for every subsequent decade of life.² Evidence indicates that stroke is taking an increasingly heavy toll on the developed world due to increases in mortality, disability and the utilization of healthcare resources.

Ischemic stroke accounts for 87% of all stroke cases and is therefore an important target for novel preventive and therapeutic strategies.¹ Ischemic stroke is secondary to the formation of a thrombus, either in the symptomatic vessel or in the form of an emboli from a more

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central source, such as the heart. Therefore, the balance between coagulation and fibrinolysis is a critical component of the disease. Thrombolysis is the only currently approved treatment for acute ischemic stroke and its effectiveness is greatly limited by common contraindications and a very narrow window of treatment. If a patient can be treated within three hours of stroke onset and has no contraindications to treatment, the intravenous tissue plasminogen activator (tPA) should be administered in an attempt to enhance clot lysis and restore blood flow to the ischemic area³. Based on the results from ECASS III, a trial that examined the efficacy of an expanded treatment window, many centers in the US have extended their treatment window to 4.5 hours, but as the drug is still only FDA approved for use within three hours of stroke onset, consent must be obtained. Beyond this time, systemic tPA increases the risk of serious side effects, including intracerebral hemorrhage.⁴ Due to this narrow treatment window, less than 5% of acute stroke patients receive thrombolytic therapy.⁵

Angioplasty for clot disruption or mechanical removal of the clot is possible, but these therapies must be performed within 8 hours of stroke onset and may only be available at advanced medical care centers.⁶ In addition, three recent randomized trials have called into question the efficacy of these therapies.^{7, 8, 9} The limitations of currently available therapies and the projected increase in stroke prevalence highlight the urgent need for further research into targeted preventive strategies and novel acute therapies that can be used to treat patients outside the current therapeutic window.

Stroke: A Sexually Dimorphic Disease

Recent research demonstrates that women are disproportionately affected by stroke. It is the 3rd leading cause of death for women, compared to the 5th leading cause of death for men¹⁰. In the United States, there are currently 26% more female stroke survivors than male and this disparity is expected to increase as the aging population continues to expand, with women increasingly outnumbering men in the elderly population.¹ This is due in part to the fact that women have a longer life expectancy, thereby increasing the incidence of stroke in the 55–75 female age group compared to their male counterparts.¹¹

Women have a lower incidence of ischemic stroke than men across most age groups, but in the highest age brackets (>85 years of age) women have higher stroke incidence.¹² In addition, women over 65 show a higher age-specific mortality rate from ischemic stroke than men.¹³ Women are also more likely to be institutionalized following stroke and have poorer post-stroke outcomes.^{14, 15} While social factors certainly play a significant role, men and women exhibit a wide variety of biological variances that may contribute to this intriguing disparity, including differences in genetics, hormonal factors and immune response.

Stroke Pathophysiology

Stroke is characterized by impaired delivery of oxygen and nutrients to brain tissues, resulting in devastating damage. The time from stroke onset to cellular death is dependent on the reduction in blood flow. The 'ischemic core' consists of tissue that receives cerebral blood flow of <10mL/100g per minute, resulting in rapid death of most cells within minutes,

whereas blood flow between 10–20mL/100g per minute characterizes the ‘ischemic penumbra’; these neurons are impaired, but still structurally intact and can regain function after blood flow has been restored.¹⁶ Oxygen and glucose deprivation in the ischemic core results in a rapid decline in production of neuronal ATP, which causes loss of the ionic gradient across the membrane and an increase in cytoplasmic Na⁺ and Ca²⁺.¹⁷ This increase elicits glutamate release, resulting in further Ca²⁺ influx through NMDA and AMPA receptors until excitotoxicity occurs, resulting in cellular degeneration and necrosis.¹⁸ Although neuronal death does not occur in the penumbra, the high extracellular levels of glutamate from the ischemic core results in increased intracellular Ca²⁺, increasing the activity of Ca²⁺-dependent enzymes that produce apoptotic mediators like superoxide, arachidonic acid and nitric oxide (NO).¹⁹

Coagulation and thrombolysis are an integral part of both the pathology and treatment of ischemic stroke. Although there is significant evidence that men and women differ in coagulability and these differences are magnified during certain female-specific life events (such as pregnancy and menopause), there is a dearth of information regarding the potential contribution of coagulation differences to the sexual dimorphism of stroke incidence and outcome. In this review we will discuss sex differences in vascular reactivity, coagulation and therapeutic interventions as they relate to stroke.

Sex Differences in Stroke: Separating Chromosomal from Hormonal

In order to fully elucidate the mechanisms behind the sexual dimorphism of stroke, it is important to recognize that males and females differ in both sex chromosome complement (XX vs. XY) **and** hormonal milieu. Although these factors are difficult to separate, it is important to recognize that sex differences in ischemic sensitivity may also be influenced by biologic sex (XX vs. XY).^{20–23}

The Dual Role of Sex Hormones

Estrogen and testosterone, the major sex hormones, have been shown to have both organizational and activational effects.²⁴ The organizational effects of hormones are permanent and independent of acute circulating hormone levels and cannot be reversed by gonadectomy. Organizational effects occur prenatally or in early postnatal life when the levels of testosterone peak in males.^{25, 26} Activational effects of hormones are dependent on the continued production of gonadal hormones and are therefore reversible by gonadectomy, and are discussed in the review by Arnold et al in this issue.

Genetic Models for the Study of Sex Differences

In order to fully examine the role that sex plays in stroke pathophysiology, it is crucial to develop a protocol that allows separation of the early organizational effects of sex hormones from those caused by sex chromosomes. In animals (and humans), the development of testis is secondary to the action of the ‘testis-determining factor’ or the *Sry* gene, which is located on the Y chromosome. When the Y chromosome is inherited, the *Sry* is inherited along with it and the animal develops testes and secretes testosterone. To specifically dissociate the organizational effects from sex chromosome effects, several investigators have capitalized

on the recently developed 'four core genotype' mouse model (^{25, 26} and see introduction in this issue). Several studies utilizing the FCG mice have successfully demonstrated the importance of sex chromosome complement to pain responses, addiction behavior and neuro-inflammation and this model may prove useful to dissociate the effects of hormones/chromosomes in the vasculature.^{25, 26,27}

X chromosome imbalance is tolerated because of dosage compensation by X-chromosome inactivation (XCI).²⁸⁻³¹ As a result of XCI, both males and females are functionally monosomic for most X-linked genes (XO). Studies in our lab using two distinct lines of XO mice found that X-chromosome dosage had no effect on stroke outcome in female mice, even when hormones were removed by ovariectomy.³² Ongoing studies in our lab utilizing the FCG mice aim to provide further information on the nature of the contribution of sex chromosomal complement to stroke sensitivity and further characterize the critical interplay between hormonal milieu and genetic sex on the pathophysiology and treatment of stroke.

Estrogen and Stroke

To date, most research on stroke risk has focused on the contribution of gonadal steroids, particularly estrogens, on ischemic sensitivity. While androgens like testosterone are believed to play an important role in cerebral vasculature, the body of work on androgens and stroke is limited and the mechanisms behind effects on pathogenesis have not yet been well elucidated. A recent comprehensive review by Gonzales highlights recent developments on this topic.³³

The neuroprotective effects of estrogen have been well characterized in animal models of stroke.³⁴ Young female animals show protection against ischemic damage when compared to their male counterparts; this effect is significantly reduced following ovariectomy (OVX) and can be restored with estrogen replacement.³⁵ Older female mice (a post-menopausal model) have been demonstrated to have greater infarct volumes and a greater inflammatory response than age-matched male counterparts and intact young females.³⁶

The majority of pre-clinical studies have focused on estrogen as the most likely cause of the lower stroke incidence seen in pre-menopausal women.³⁷ But contrary to evidence found in experimental animal studies and observational cohort studies, large controlled clinical trials analyzing the use of estrogen in the primary and secondary prevention of stroke have proven unsuccessful.^{38,39} Interestingly, clinical evidence seems to suggest that hormone replacement therapy (HRT) actually increases stroke risk (see below).⁴⁰

Sex Differences in the Cerebral Vasculature

Sex Hormones and Vascular Reactivity

Impaired cerebrovascular activity results from the accumulation of vascular pathology such as loss of compliance, inflammation, endothelial damage and atherosclerosis and has been shown to be associated with an increased risk of ischemic events.⁴¹ Vascular reactivity is characterized as the ability of the cerebral arterioles to improve cerebral blood flow by dilating in response to hypercapnia.⁴² Studies using transcranial Doppler as a measure of

vasomotor reactivity found that impairment of normal reactivity was associated with a greater risk of stroke in the setting of occlusive carotid artery disease.^{43,44}

The strong association between vascular reactivity and stroke risk may provide one mechanism by which sex hormones affect the risk of ischemia in the cerebral vasculature. Sex hormones, particularly estrogen, have been demonstrated to have a profound influence on stroke pathophysiology and cerebrovascular reactivity. Studies have shown that estrogen stimulates the production of NO and that synthesis and release of NO in response to acetylcholine is higher in females than in males, which may contribute to differences in vascular function between sexes.^{45–48} Cerebral blood flow is 'autoregulated' to protect the brain from variations in blood flow and pressure; it is believed that NO-dependent mechanisms, like myogenic tone, make an important contribution to that process. Female mice and ovariectomized (OVX) mice with 17- β estradiol (E2) replacement were shown to have lower myogenic activity than their male or OVX counterparts.⁴⁹

Estrogen interacts primarily with cerebral endothelium and, to a lesser extent, vascular smooth muscle, to elicit both genomic and non-genomic effects. Nuclear estrogen receptors (ERs) mediate the genomic effects of estrogen and are expressed in the nucleus, plasma membrane, and mitochondria of endothelial and smooth muscle cells of cerebral arteries; activation and conformational change of ERs can result in various effects, including changes in downstream signaling and target gene transcription.⁵⁰ The plasma membrane of vascular cells also expresses a moderate amount of ER and other estrogen-responsive membrane proteins, allowing estrogen to exert non-genomic effects resulting in increased cerebral blood flow and decreased cerebral vascular tone.⁵¹ These vasodilatory effects are mediated via augmentation of the prostacyclin and endothelial-derived nitric oxide pathways.⁵²

The effect of estrogen on nitric oxide (NO) has been shown to play a significant role in the sex-hormone dependent differences in vascular reactivity. Estrogen increases the synthesis and activity of eNOS, the endothelial enzyme responsible for NO production, by a variety of mechanisms. Stimulation of nuclear estrogen receptors (ER) results in increased eNOS expression and in vitro incubation with E2 has been shown to increase protein levels of eNOS.^{53,54} Cerebral blood vessels from mice and rats exposed to estrogen were shown to have higher levels of eNOS mRNA and protein, an ER- α dependent effect seen in both sexes.^{55–57} Estrogen also influences NO synthesis in a non-genomic manner by activating the phosphoinositide-3 kinase-Akt (PI-3k/Akt) pathway, resulting in phosphorylation of eNOS; this modification increases both its activity and sensitivity to calcium stimulation.⁵⁸

Estrogen has been demonstrated to have significant effects on mitochondrial function, including a suppression of ROS production in cerebral blood vessels and endothelium.^{59,60} In addition, in vivo and in vitro estrogen treatment resulted in increased protein levels of electron transfer chain proteins and critical enzymes in the citric acid cycle.⁶¹ These results suggest that the presence of estrogen augments mitochondrial function while simultaneously suppressing potentially harmful ROS production, a finding that is particularly important in an area of high metabolic function like the cerebral vasculature.⁶²

Sex hormones have also been shown to affect vascular reactivity by altering the production of cyclooxygenase-dependent prostanoids.⁵² Estrogen alters the balance of prostanoids by enhancing the production of vasodilating prostacyclin (PGI₂), whereas testosterone favors vasoconstriction through an increase in thromboxane (TxA₂) activity.^{63,64} Estrogen-mediated increases in PGI₂ have been demonstrated as a result from increases in two enzymes, cyclooxygenase-1 (COX-1) and PGI₂ synthase.⁶⁵ Male rats treated with testosterone were found to have greater constriction of cerebral arteries and higher levels of TxA₂ synthase in their cerebral vessels, an effect that was abolished by treatment with TxA₂ synthase or thromboxane receptor inhibitors.⁶³ The reader is directed to several comprehensive reviews on this topic.^{52,66}

These results indicate that sex hormones, particularly estrogens, play an integral role in the regulation of vascular reactivity, contributing to better tissue perfusion in females during and after occlusion of cerebral arteries which could contribute to reduced stroke volumes.

Sex differences in Endothelial Cells

Recent work in cultured endothelial cells derived from male or female embryonic mice have shown a differential sensitivity to ischemic injury, with intrinsic female protection, similar to what has been reported in astrocytes and neurons.^{67–69} As these studies utilized embryonic cells or neonatal brain slices cultured in steroid free media, factors besides acute hormone exposure must be contributing. However the etiology of the differential sensitivity of male and female cells is unknown. In endothelial cells differences in epoxyeicosanoid signaling appear to be an important contributor to ischemic sex dimorphism.

Epoxyeicosatrienoic acids (EETs) are arachidonic acid metabolites of cytochrome P450 (CYP) epoxygenases produced in the brain by astrocytes and vascular endothelium. EETs protect the brain from ischemic injury and contribute to vasodilation. They are metabolized by the enzyme soluble epoxide hydrolase (sEH).^{70,71} Female derived cerebral microvessels have lower sEH expression and higher EETs levels after oxygen-glucose deprivation, which may contribute to the differential resistance seen in female cells after ischemic injury.⁶¹ In vivo, the cerebrovascular expression and activity of sEH was lower in females compared to males.⁷² Cerebral blood flow (CBF) during middle cerebral artery occlusion (MCAO) was higher and infarct size was smaller in females compared with males, although not all studies have found a consistent sex difference in CBF in this stroke model.²¹ These sex differences were absent in sEH knockout mice. Importantly, the sex differences in CBF and ischemic injury were abolished after ovariectomy, suggesting a complex interaction with ovarian steroids in vivo.

Coagulation Factors, Sex and Stroke

When vascular injury occurs, blood platelets and coagulation mediators are activated and cause the formation of a fibrin-platelet clot. While the serine protease cascade of blood coagulation is crucial for limiting excessive blood loss during trauma, it also plays an adverse role in disorders of acute thrombosis including ischemic stroke. The classic coagulation cascade can be activated by two serine protease pathways, both of which result in the formation of thrombin, which can then convert fibrinogen to fibrin and enable clot formation to occur.

Intrinsic Coagulation Cascade in Stroke

The extrinsic pathway is induced when tissue factor comes into contact with activated factor VII (FVIIa).⁷³ In contrast, the intrinsic pathway is initiated when factor XII (FXII) becomes autoactivated by binding negatively charged surfaces.⁷⁴ Historically, it was believed that the extrinsic pathway was primarily responsible for fibrin formation because hereditary deficiencies in the extrinsic pathway (FVII and tissue factor) resulted in excessive bleeding, whereas deficiency in FXII was not shown to have the same association.^{75,76} However, recent studies showed that mice deficient in FXII are protected in models of ischemic stroke without significant variations in hemostasis, suggesting that hormone-mediated effects on factor FXII should be investigated as a potential mechanism for female neuroprotection.^{77,78}

Recent experiments by Hagedorn and colleagues showed that an insect-derived inhibitor of activated FXIIa abolished platelet aggregation and thrombus formation at vascular injury sites, as well as protecting against neurological damage in a mouse model of transient cerebral ischemia.⁷⁹ The second Northwick Part Heart Study, conducted in middle-aged men, showed that lower levels of FXII inhibitory complexes were associated with an increased risk of coronary heart disease (CHD) and stroke.⁸⁰ Estrogens are believed to have an impact on the transcriptional regulation of coagulation protein genes; in particular, the gene for FXII (F12) has an estrogen receptive element in its promoter region.^{81,82} Investigators in the RATIO study examined a young female cohort to determine whether estrogen altered the relationship between intrinsic pathway factors and arterial thrombosis. They found that high levels of activated intrinsic system proteins are associated with increased risk of ischemic stroke, but did not significantly increase the risk for myocardial infarction. In addition, oral contraceptive use further increased the risk of ischemic stroke, but was not significant enough to contraindicate OC use in this population.⁸³

Given recent evidence, it appears that inhibition of coagulation factors of the intrinsic pathway like FXIIa provide a novel and potentially powerful target for primary prevention of stroke. In addition to the estrogen sensitivity of F12 gene transcription, a recent study in mice suggests that estrogen may also play a role in the transcriptional regulation of other coagulation proteins.⁸² Rio-Espinola and colleagues report that the rs1801020 SNP, which increases FXII activity, is associated with reduced adverse side effects following tPA treatment for acute ischemic stroke.⁸⁴ When exploring inhibitory treatment of intrinsic pathway proteins, it will be necessary to determine whether there is a sexual dimorphism in baseline production of these factors and whether differential dosing is required based upon estrogen levels.

Plasminogen Activating Inhibitor and Stroke

Epidemiological evidence suggests that there may also be sexual dimorphism in coagulation and fibrinolytic factors in patients with acute ischemic stroke. Although PAI-1 levels have been found to be similar in healthy males and females, studies done in coronary stenosis and type 2 diabetes patients have reported higher PAI-1 levels in females compared to males.⁸⁵⁻⁸⁷

In 2003, Kain and colleagues found significantly higher levels of PAI-1 antigen, FVII:C and FVIII:C levels and lower fibrinogen levels in female stroke patients than in their male counterparts.⁸⁸ The authors report that majority of their female subject were postmenopausal and elderly, with a low likelihood of currently receiving hormone replacement therapy (HRT). Previous studies have shown that healthy females receiving HRT or with high estrogen levels have lower levels of PAI-1 than low-estrogen counterparts.⁸⁹ These results may indicate that the presence of estrogen suppresses PAI-1 levels in pre-menopausal women and contributes to the heightened risk of ischemic stroke seen in post-menopausal women.

C-Reactive Protein and Stroke

One of the most common causes of stroke is atherosclerosis, a chronic inflammatory state resulting from injury to the endothelial surface.⁹⁰ C-reactive protein (CRP) is a marker of the acute inflammatory response and contributes to the release of chemokines and thrombogenic factors. An association between elevated CRP levels and stroke has been well documented and there appears to be an increased risk for the female sex; elevated CRP levels cause risk of stroke to double in men and to triple in women.^{91,92} In a comparison between the highest and lowest quartile for CRP, the age-adjusted risk for first ischemic stroke/TIA was 2.7 for women (P=0.0003) and 2.0 for men (P=0.027).⁹³

Platelets, Sex and Stroke

Estrogen, Testosterone and Platelet Reactivity

Platelets play a critical role in atherosclerosis and subsequent thrombus generation. Platelets can be activated by circulating factors in the blood or by the sub-endothelial collagen exposed during endothelial injury. Following the rupture of an atherosclerotic plaque, platelets adhere to exposed collagen and von Willebrand factor (vWF) and are bound by thrombin, resulting in platelet activation. This activation is characterized by the secretion of several agonists; dense granules release adenosine diphosphate (ADP) and platelet cyclooxygenase-1 (COX-1) converts phospholipid-derived arachidonic acid to prostaglandin PGH₂, which is then converted to TxA₂ by platelet-specific thromboxane synthase and released, resulting in amplification of the platelet activating signal and induction of platelet aggregation at the site. Generation of a stable thrombus is also contingent upon sustained activation of the GPIIb/IIIa receptor, requiring continuous ADP-P27₁₂ signaling. Activation of platelets also exposes phosphatidylserine at their surface, allowing coagulation factors to bind and produce thrombin in large quantities, facilitating the formation of a stable fibrin/platelet clot at the site.

The effect of sex steroids on platelet response remains controversial, due in part to the fact that some studies utilize in vivo models to examine genomic sex steroid effects in vivo, while others focus on the in vitro effects resulting from the addition of hormone to platelets. An in vivo study with rats reported that male rats showed increased thrombus formation when compared to females; 6 weeks of treatment with synthetic estrogen significantly reduced thrombotic deposits in both sexes.⁹⁴ A study involving thrombosis in rats revealed that thrombus size and mortality rate in males were double that of females, and

administration of testosterone worsened these effects for both sexes. When testosterone-treated rats received anti-androgen treatment, the mortality rate was significantly decreased in both male and female rats.⁹⁵

In further experiments, platelets isolated from male and female rats were found to have similar intrinsic sensitivity to ADP, collagen and arachidonic acid. However, platelets from male rats were more sensitive to inhibition by PGI₂ and less responsive to thrombin than female platelets; male rats also showed plasma fibrinogen levels almost twice those of female rats. After 6 weeks of estrogen treatment to mimic oral contraceptive use, platelets from female rats showed a decrease in collagen-induced ATP release and aggregation without any other changes in platelet reactivity.⁹⁴

Conversely, experiments done by Leng and colleagues demonstrated that platelets isolated from female mice showed a higher intrinsic tendency to bind fibrinogen in response to low concentrations of collagen-related peptide (CRP) and thrombin and had a greater aggregation response to ADP and CRP than those isolated from males.⁹⁶ Another in vitro study demonstrated that male rat platelets were 10 times more responsive to ADP than platelets from female rats; castration reduced the ADP response in males and ovariectomy enhanced responsiveness in females and testosterone enhanced platelet response in both sexes. Platelets isolated from both rats and guinea pigs showed an enhanced platelet response when incubated with testosterone, an effect that was antagonized by flutamide (an androgen receptor antagonist) and estradiol.⁹⁷

Jayachandran et al examined porcine platelet reactivity as a potential mechanism behind the differences in vasoprotection following estrogen loss.⁹⁸ Four weeks after ovariectomy, platelet turnover, aggregation and ATP secretion were increased. They also demonstrated alterations in matrix metalloproteinase levels, which are involved in vascular remodeling in response and are known to be regulated by sex steroids in reproductive tissues. These changes, in particular the up regulation of MMP-2, may contribute to an enhanced response to vessel injury following menopause and could facilitate the development of coronary artery disease and vascular dysfunction.⁹⁸

Although intrinsic differences in the reactivity of male and female platelets have yet to be fully elucidated, these experiments suggest that testosterone augments platelet responsiveness and promotes prothrombotic states, whereas estrogen attenuates the prothrombotic state in platelets. Further elucidation of the mechanisms by which sex steroids, particularly estrogen, impact platelet reactivity is crucial in the further refinement of the cornerstone of primary and secondary stroke prevention: antiplatelet therapies.

Platelet activation and aggregation play a critical role in thrombus generation. It has been suggested human platelet reactivity may be influenced by sex and sex hormones; both men and women express estrogen receptors on their platelets.⁹⁹ This relationship appears to be complex; while women appear to have greater baseline platelet reactivity (Table 1), estrogen may play a role in attenuating this increased activity and may be part of the reason why stroke incidence in women increases post-menopause. Platelets isolated from post-

menopausal women and exposed to E2 in vitro have been shown to have decreased ADP-induced aggregation and ATP release.¹⁰⁰

Antiplatelet Therapy and Stroke

There are three commonly used antiplatelet strategies; inhibition of the GPIIb/IIIa receptor, inhibition of the ADP-P2Y₁₂ interaction and inhibition of COX-1 by aspirin. Currently, aspirin represents the leading anti-platelet strategy for both primary and secondary prevention. The Women's Health Study, a large primary prevention study, showed that aspirin therapy significantly reduced stroke incidence (RR = .84, 95% CI = .69–99), but did not reduce likelihood of MI and death.¹⁰¹ A meta-analysis of 6 primary prevention trials showed a 12% reduction in vascular events with no significant differences based on sex, although there was a trend towards aspirin therapy being more effective in primary prevention of ischemic stroke in women (P=.08).¹⁰² Another subsequent meta-analysis (n=95,456) reported a 17% reduction in ischemic stroke rate in women following aspirin therapy (P=.02).¹⁰³

Recently, the use of dual antiplatelet therapy with aspirin and clopidogrel, a thienopyridine derivative that inhibits ADP-P2Y₁₂ interaction has become standard for primary prevention of thrombosis in patients receiving coronary stent implantation (CSI) or presenting with acute coronary syndrome (ACS).^{104,105} Meta-analysis of large clinical studies showed that dual therapy significantly reduced total ischemic events compared to aspirin alone, and that adding clopidogrel therapy led to a significant reduction of stroke in men, the effects on stroke were not significant in women. In addition, dual therapy resulted in a 43% increased rate of major bleeding in women, compared to a 22% increased rate in men.¹⁰⁶ Recent trials indicate that women tend to be at greater risk of primary end-point occurrence than men, regardless of type or dosage of P2Y₁₂ receptor therapy.¹⁰⁷ The mechanism behind this increased risk remains to be elucidated; it may be a result of reduced antiplatelet response, dosage or metabolism issues or higher platelet reactivity or a combination of these factors.

Hypercoagulability and Stroke

Ischemic strokes can be thrombotic or embolic¹⁰⁸. Virchow's triad describes the three triggers for thrombosis; hypercoagulability, endothelial injury and hemodynamic stasis. The most common pathophysiology that involves this triad is atherosclerosis (endothelial injury). In some cases, the vessel wall will break down and become damaged, resulting in the release of pro-coagulant and pro-thrombotic factors.¹⁰⁹

While hypercoagulable states have been implicated as a risk factor for stroke, there is not enough data to make specific primary prevention recommendations. The majority of acquired and hereditary thrombophilias are not associated with increased risk of cerebral infarction.¹¹⁰

Acquired Hypercoagulable States

The presence of antiphospholipid antibodies (aPLs) shows the strongest association with arterial thrombosis, as measured by anticardiolipin antibodies (aCLs) and lupus anticoagulant (LA) levels.¹¹⁰ aCLs are believed to promote arterial thrombosis through their

activation of endothelial cells, resulting in up-regulation of pro-inflammatory cytokine secretion, tissue factor release and initiation of the coagulation cascade.¹¹¹ These changes, in combination with endothelial dysfunction, can promote formation of atheromatous lesions over time.¹¹²

Past retrospective studies have noted an association between ACLs and ischemic stroke. The Antiphospholipid Antibody and Stroke Study (APASS) found that aCL antibodies were found significantly more often in patients presenting with acute stroke than in controls and correlated with worse cognitive impairment post-stroke, but did not affect the rate of stroke recurrence or mortality at 30 days or 1 year.^{113,114} Two subsequent studies in elderly populations also reported no association between aCL titers and stroke recurrence.^{115,116} Although APASS reported no differential response to aspirin versus warfarin in prevention of stroke recurrence in patients with elevated aCL titers, subsequent retrospective series showed that aspirin alone has minimal effect on preventing recurrence in these patients.^{117,118} Warfarin is the current recommendation for management of non-pregnant aPL positive patients with a history of past thrombotic events.¹¹⁹

The Framingham Offspring Cohort Study reported a sex-specific association between aCL titers and ischemic stroke or transient ischemic attack (TIA). This study demonstrated that age increases the likelihood of testing positive and that women with an aCL SR above the threshold of 0.4 h were at increased risk for ischemic stroke and showed a significantly higher relative risk for ischemia stroke/TIA than their male counterparts.¹²⁰ Interestingly, there was a trend towards higher relative risk in pre-menopausal and peri-menopausal women above the aCL threshold than in post-menopausal women.¹²⁰ Experimental animal studies have shown increased aCL induction in female mice compared to male mice and this is thought to be secondary to estrogen.¹²¹ A case-control study in young women (15–44 years) also found significant association between aCL and LA levels and stroke incidence.¹²² These results indicate that although estrogen itself has been shown to neuroprotective, it may also play a role in the induction of aPL, atheromatous changes, and hypercoagulability leading to an increase in stroke risk, especially at supra-physiological levels.

Sex-Specific Coagulation Risk Factors

With the exception of the very elderly (>85) years, men are at a higher risk of stroke than women across all ages. However, there are two conditions unique to women that cause stroke incidence to spike – pregnancy/peripartum (19–30 years of age) and the peri-menopause (45–54 years of age).^{123,124} Many of these “sex-specific” factors have been discussed in depth in a recent guideline released by the American Heart Association that focused on stroke prevention in Women.¹⁰

Pregnancy and Oral Contraception

A recent survey by the Department of Health and Human Services indicated that 10.7 million women in the US (age 15–44) use oral contraceptives.¹²⁵ Although the risk of stroke in this age group is very low, it is thought that oral contraceptive use may represent a significant risk factor for ischemic stroke in this age group. Although this topic is still

controversial, there does seem to be a slight increase in risk of ischemic stroke with OC use, an effect that is mitigated by using an intermediate dosage of ethinyl estradiol or the use of progestin-only OC.¹²⁶ There is also evidence that OC use magnifies the ischemic stroke risk associated with hypercholesterolemia, obesity and other thrombotic risk factors.¹²⁷

Interestingly, several studies have shown that progestin only formulas do not show an association with increased stroke risk.^{128,129} A population-based cohort study of different hormonal contraception formulations in Denmark also reported that progestin-only and intermediate dosage ethinyl estradiol OCP were not associated with a risk of ischemic stroke, whereas the transdermal and vaginal rings were associated with a similar risk as high dose ethinyl estradiol pills.¹²⁶

Although stroke is not common during pregnancy, the risk for stroke is higher in pregnant women (34 per 100,000 deliveries) than in non-pregnant women (21 per 100,000).^{126,130} The leading cause of ischemic stroke in pregnancy and postpartum women is hypertension related to pregnancy, including pre-eclampsia/eclampsia.¹³¹ A history of pre-eclampsia increases the risk of stroke in patients even after their childbearing years are over; early onset preeclampsia (before 32 weeks) is associated with a five-fold increase in stroke risk over general preeclampsia.¹³² In addition, it has been found that offspring resulting from pre-eclamptic pregnancies have a higher stroke risk as adults, indicating that there may be a maternal-fetal interaction involved in stroke pathogenesis.¹³³ Pregnancy also results in a hypercoagulable state due to decreased levels of protein S, increased fibrinogen, increased plasminogen activator inhibitor type 1 levels (PAI-1) and activated protein C resistance.¹³⁴ These changes begin at conception and do not return to baseline until approximately two months post-partum, reflecting the fact that the highest stroke risk occurs in the third-trimester and continues into the puerperium.^{130,134}

Menopause and Hormone Replacement Therapy

There is evidence that estrogen plays a significant role in the decreased stroke risk seen in pre-menopausal women; the risk of stroke in women increases after the onset of natural or surgically induced menopause.^{135,136} While some studies suggest that age of menopause onset has an effect on stroke risk, data has been inconsistent and further investigation is required to determine the nature of this complex relationship.¹³⁶ Although estrogen supplementation in post-menopausal animal models was found to be protective against stroke, 29 observational studies have found no conclusive evidence that hormone replacement therapy (HRT) has a similar effect in humans.¹³⁷ Two large clinical trials subsequently performed in women with a history of vascular disease found that stroke occurrence was not decreased in women who received estrogen compared to placebo.^{39,138} The randomized WHI trial involved older women than previously studied (mean age = 63) without a history of vascular disease and found that the risk of ischemic stroke with estrogen supplementation alone actually increased (HR = 1.55 95% CI 1.10–2.01).¹¹⁰ Subsequent studies and meta-analysis have shown that HRT does not reduce stroke occurrence in women and may actually result in increased stroke risk.¹³⁹

One of the major limitations in the WHI design was that treated women were much older and were less healthy than women in prior observational cohorts that showed a benefit of

hormone therapy. Many had been without estrogen for as long as a decade prior to randomization. This raised the possibility that the long period of hypoestrogenicity or advanced age in treated women confounded the results. Subsequent animal studies demonstrated that estrogen has a pro-inflammatory effect in aged female mice, and exacerbated ischemic damage consistent with what was seen in elderly women.^{140,141} However, emerging follow-up studies analyzing the WHI cohort (see review by Howard et al, 2013) have demonstrated that the adverse effects of hormone replacement therapy extend to all women, regardless of ethnicity, risk factor profile (obesity, diabetes etc.), medication use (statin or aspirin use) or age.¹⁴² Younger women also had an increased risk for stroke, venous thromboembolism and peripheral artery disease with treatment. This risk is attributable to exogenous hormone exposure, as analyses of discontinuation data in these trials suggest that the adverse effects of hormone therapy on vascular risk are reversible.^{143,144}

While the exact mechanisms of sex in stroke pathophysiology have yet to be elucidated, it is clear that estrogen balance is a key factor. Further understanding of this complex homeostasis will allow the development of therapies that maximize estrogen's neuroprotective effects while minimizing the associated risk of increased thrombogenesis.

Sex and Venous Thrombosis

Interestingly, although the risk of a first venous thrombosis is thought to be equal among sexes, recurrent venous thrombosis has been demonstrated to occur at a higher rate in men (approximately 10% per year) than in women (2–5% per year).^{145,146} A recent study found that a similar sex difference exists for first venous thrombosis rate, but is masked by female-specific risk factors such as pregnancy, HRT and oral contraception.¹⁴⁷ Clinical trials have demonstrated HRT may escalate stroke risk via an increased risk of venous thrombosis.^{148,149} These results indicate a potentially important link between estrogen and increased thrombus generation and underline an important paradox; while estrogen is neuroprotective in the brain, it may contribute to the pathogenesis of stroke via the induction of a pro-thrombotic state.

As mentioned previously, the use of HRT and estrogen-containing OCP are also associated with an increased risk of venous thromboembolism, including deep vein thrombosis and more serious conditions like pulmonary embolus and cerebral venous thrombosis¹⁵⁰. Importantly, Holmegard et al demonstrated that high endogenous levels of the sex hormones testosterone and estradiol are not associated with an increased risk of venous thromboembolism.¹⁵¹

Thrombolysis, Sex and Stroke

Sex and tPA Efficacy

There is some evidence that women are less likely to receive tPA thrombolysis than men, due in part to differences in stroke symptomology and presentation between men and women although these differences have not been found in more recent studies.¹⁵² Several studies have also examined whether sex plays a role in the physiologic response to

thrombolytic treatment with mixed results. Evidence has shown that in patients who do not receive thrombolytic therapy, women have poorer functional outcomes than men.¹⁵³

In an analysis of the PROACT-2 Study, Hill and colleagues found that while intra-arterial tPA treatment resulted in better outcomes in both sexes, there was a greater absolute benefit in women (20%) than in men (10%).¹⁵⁴ Pooled analysis of five randomized control trials showed that intravenous rtPA treatment also eliminates the outcome disparity between sexes, indicating that women may derive a more significant benefit from tPA therapy than men.¹⁵⁵ Further investigated into this nullification effect in a large registry of rtPA-treated stroke patients found no significant sex differences in functional outcomes 90 days post-stroke.¹⁵⁶

These studies seem to suggest that while women suffer from worse functional outcomes than men, the administration of both IV and intra-arterial tPA has an greater beneficial effect in women, resulting in an amelioration of the sex disparity in stroke outcomes. Savitz et al demonstrated that this effect may be due, in part, to the fact that women exhibit an increased recanalization rate following IV rtPA administration compared to men.¹⁵⁷ This increased recanalization response in women is believed to be due to reduced arterial size in women (resulting in smaller clots), in addition to the vast array of sex differences in coagulation and fibrinolysis discussed earlier in this review.^{88,158,159}

The Metabolic Syndrome and Acute Ischemic Stroke

Obesity is becoming a common problem in the US. This is particularly concerning because the risk of many serious diseases, including stroke, increases dramatically with obesity and its associated complications. Insulin resistance associated with obesity and poor diet contributes to a common set of vascular risk factors known as the Metabolic Syndrome (MetS) (central obesity, high blood pressure, dyslipidemia and hyperglycemia) that is associated with an increased risk for ischemic stroke.^{160,161} It is thought that the increased risk may be due to the fact that MetS causes disruptions in endogenous fibrinolysis, enhancing PAI-1 levels and increasing the risk of thrombotic events.^{162,163}

A population-based prospective study demonstrated that MetS has a greater impact on adverse vascular events in women than in men, including higher risk of ischemic stroke.¹⁶⁴ Due to the impairment of fibrinolysis seen in these patients, it has been suggested that MetS might increase resistance to tPA administration and reduce the efficacy of clot lysis.¹⁶⁵ Arenillas and colleagues showed that this effect did exhibit sex differences in the treatment of acute middle cerebral artery ischemic stroke with tPA; MetS seemed to increase resistance to lysis in women more than in men.¹⁶⁶ This suggests that the increase in obesity and resulting MetS in the United States may contribute to increased stroke risk in both sexes, but may have a particularly negative effect on both the pathogenesis and treatment of stroke in women.

Conclusion

The concept of “individualized medicine” is at the forefront of current medical innovation. Yet sex differences, arguably the most fundamental and significant variable in personalized

risks and treatment, have yet to be fully examined. In order to deliver the best possible medical care for an individual with a life-threatening condition like ischemic stroke, research must fully elucidate the mechanisms by which sex chromosomes, hormonal status and lifestyle factors impact the incidence and outcome of this debilitating disease. The final goal of “sex-based” research is to determine whether the pathophysiology of stroke truly differs in men and women – and how to translate that knowledge into specific and effective strategies for prevention, diagnosis and therapy.

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Figure 1. Cerebral Vasodilatory Estrogen Effects

Sex differences in the regulation of cerebral vasculature have been described. Prolonged exposure to estrogen promotes cerebral vasodilation by increasing vasodilatory factors and suppression of vasoconstriction mechanisms; these effects are mediated via both genomic and non-genomic pathways.

Figure 2. Simplified schematic of significant effects of estrogen on coagulation

Estrogen influences the levels of many clotting factors via gene transcription, increasing the likelihood of clotting and related complications, including stroke. The most significant increase involves factor VII of the extrinsic coagulation cascade. While reduction in FVII results in excessive bleeding, reduction of FXII, another estrogen-increased clotting factor does not appear to have the same bleeding risks and may prove a novel target for stroke prevention, particularly in women.

Table 1**Human Studies on Sex Differences in Platelet Reactivity in Healthy Volunteers**

Author	Study Population	Findings
Faraday et al (1997)	Healthy volunteers	<ol style="list-style-type: none"> 1 No difference in total # of GPII/IIIa receptors; women had ↑ number of activated GPII/IIIa receptors per platelet 1 GPIIb/IIIa reactivity varies with menstrual cycle
Kurrelmeyer et al. (2003)	Asymptomatic volunteers (n=400) with family hx of premature CAD	<ol style="list-style-type: none"> 2 Platelets from women: <ul style="list-style-type: none"> • ↑ Fibrinogen binding upon ADP exposure • ↑ Spontaneous aggregation 3 ↑ plasma thromboxane B₂ levels in women
Yee et al (2005)	Healthy volunteers (n=359)	<ol style="list-style-type: none"> 1 Small subset was hyperresponsive (< 65% max platelet aggregation) to ristocetin, ADP, collagen, epinephrine, CRP 2 Hyperresponsiveness associated with female sex
Becker et al (2006)	Asymptomatic volunteers (n=1,282) with family hx of premature CAD	<ol style="list-style-type: none"> 1 Women's platelets ↑ to 10 platelet agonists at baseline 2 Aggregation to the direct COX-1 pathway, arachidonic acid (AA), ↓ more in women than men following aspirin therapy 3 Women experienced similar or ↑ inhibition of platelets in COX-1 indirect pathways after aspirin therapy,
Santimone et al (2011)	Cohort study (n=18,1907)	<ol style="list-style-type: none"> 1 ↑ Platelet incidences in women 2 ↑ Platelet count and plateletcrit associated with inflammatory indices (WBC count, CRP and D-dimer levels) 3 Sex differences regardless of age and menopausal status

Table 2

Summary of large meta-analysis or cohort studies investigating risk of ischemic stroke associated with pregnancy and oral contraceptive (OC) use.

Author	Study Design	Finding
<i>Oral Contraceptives and IS Risk</i>		
Gillum et al (2000)	Meta-analysis; any OC use	OR = 2.75 (95% CI 2.24 – 3.38)
Chan et al (2004)	Meta-analysis; any OC use	OR = 2.13 (95% CI 1.59 to 2.86) (case-control only) for IS
Baillargeon et al (2005)	Meta-analysis; low dose combined OC	OR = 2.12 (95% CI 1.56 to 2.86)
Chakhtoura et al (2009)	Systematic review; progestin-only OC	No significant increased risk
Yang et al (2009)	Swedish cohort study (n=49,259)	No significant increased risk
Lidegaard et al (2012)	Danish cohort study (n=1.6 million)	Ethinyl Estradiol 30–40 microgram dosage: ARR = 1.40 (95% CI 0.97–2.03) to 2.20 (1.79–2.69) Ethinyl estradiol 20 microgram dosage: ARR = .88 (.22–3.53) to 1.53 (1.26–1.87) No significant increased risk with progestin only formulas
<i>Pregnancy/Puerpium and IS Risk</i>		
Bellamy et al (2007)	Meta-analysis; history of preeclampsia	OR = 1.81 (95% CI 1.37 – 2.33) for any stroke
Brown et al (2013)	Meta-analysis; history of preeclampsia	OR = 1.76 (95% CI 1.43 – 2.21) for any CVD
McDonald et al (2010)	Meta-analysis; history of preeclampsia	OR = 2.03 (95% CI 1.54 – 2.67) for any CVD
Smith et al (2012)	Meta-analysis; history of preeclampsia	OR = 3.25 (95% CI 1.76–7.11) for lifetime risk of any CVD