

## Biphasic effects of hormone treatment on risk of cardiovascular disease

### RESOLVING THE PARADOX IN POSTMENOPAUSAL WOMEN

► S. Mitchell Harman, MD, PhD, and Eliot A. Brinton, MD, FAHA, FNLA

**C**ardiovascular disease (CVD) caused by atherosclerosis remains the greatest single killer of women, accounting for approximately 40% of total mortality.<sup>1</sup> The current armamentarium of preventive measures against CVD include maintenance of a healthy lifestyle, notably smoking cessation, maintenance of a healthy body weight and regular exercise,<sup>2-4</sup> control or prevention/treatment of hypertension,<sup>5,6</sup> and appropriate use of medications that lower low-density lipoprotein

cholesterol (LDL-C) and raise high-density lipoprotein cholesterol (HDL-C).<sup>7,8</sup> However, lifestyle interventions that are effective in highly structured research studies are difficult to sustain in clinical practice. For example, there are high rates of recidivism even after initial success with smoking cessation<sup>9</sup> or weight loss programs.<sup>10</sup> Anti-hypertensive and lipid-lowering medications may be expensive and have a variety of adverse effects, and noncompliance is often an issue. Moreover, approximately half of patients with a first CVD event would not have been candidates for lipid-lowering medications based on current guidelines for LDL cholesterol-lowering medication, which has led to recommendations for reducing the target LDL level.<sup>11</sup> Further, lipid medica-

tions allow a majority of CVD events to occur.<sup>12</sup> Given the above, the problem of CVD prevention in older women remains a pressing, unresolved issue.

Regularly ovulating women of reproductive age are significantly protected against CVD compared with men, but CVD increases within 10 years of menopause to levels matching or exceeding incidence rates among men,<sup>13</sup> suggesting that ovarian estrogen may help protect women against atherosclerosis. This observation led to the corollary hypothesis that treatment of postmenopausal women with estrogens (menopausal hormone therapy [MHT]) might prevent the postmenopausal increase in CVD rates. Yet, despite more than 30 years of research attempting to define whether and how postmenopausal estrogen replacement might protect women against CVD, the issue remains uncertain and, indeed, controversial.

#### Progression of atherosclerosis

Atherosclerosis is a progressive pathological process characterized by the formation of plaques in the arterial wall, typically at sites of endothelial injury, frequently at arterial branch

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S. Mitchell Harman, MD, PhD  
Kronos Longevity Research Institute  
Phoenix, Arizona

Eliot A. Brinton, MD, FAHA, FNLA  
Metabolism Section, Cardiovascular Genetics  
University of Utah College of Medicine  
Salt Lake City, Utah

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**Nanette F. Santoro, MD**  
Professor and Director  
Division of Reproductive Endocrinology  
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Bronx, New York

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**American Society for Reproductive Medicine**  
1209 Montgomery Hwy., Birmingham, AL 35216  
(205) 978-5000 • asrm@asrm.org • www.asrm.org

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## Something old, something new

Women, hormones, and heart disease. The debate rages on. This issue of *Menopausal Medicine* revisits the question

of cardiovascular benefit for estrogen use in the early postmenopausal years. A scholarly discussion of why the effect of hormones on the heart must be examined further is provided by Dr S. Mitchell Harman, director and president of the Kronos Longevity Research Institute and principal investigator of the Kronos Early Estrogen Prevention Study (KEEPS), and Dr Eliot A. Brinton, scientific advisor, Kronos Longevity Research Institute.

It is important to reconcile the discrepancies that currently exist between observational studies of hormone use and cardiovascular disease—which showed substantial benefit—and the WHI randomized, clinical trial, which did not demonstrate any benefit. KEEPS is designed to test the most promising hypothesis to emerge after the unexpected WHI findings. The “timing” hypothesis states that hormones will have maximum benefit in a setting where vessels are minimally besieged with plaque. For most women, the opportunity to intervene is in the early postmenopausal years. Could this be why the WHI did not demonstrate coronary disease prevention? Time will tell.

Drs Lubna Pal and Susan M. Hailpern raise the issue of hormones and heart disease earlier in life. Can it be that premenopausal women with reduced ovarian reserve are at greater risk for heart disease? Based on known risk factors, this would appear to be the case. But which comes first? Does the heart disease precede the ovarian failure or vice versa? You will have to read on for the full discussion.

This is how science advances. Each piece of new data builds upon prior studies, and hopefully, we move the research, and ultimately, the care of our patients, in the healthiest direction.

**Nanette F. Santoro, MD**

points where laminar blood flow is interrupted and turbulence occurs.<sup>14</sup> This phenomenon is intensified by high blood pressure. Impaired endothelial function (EF) is believed to be an important etiological factor contributing to the pathogenesis of atherosclerosis.<sup>15</sup> Flow-mediated (post-occlusion) arterial vasodilation (FMD) is a compensatory vascular reaction to a period of ischemia and is mediated mainly by effects of nitric oxide (NO) released by the endothelium, which relaxes arterial smooth muscle.<sup>16</sup> Numerous observations support the conclusion that impaired FMD is a strong predictor of both prevalent<sup>17-19</sup> and incident<sup>20,21</sup> CVD. Injured endothelium facilitates the infiltration of atherogenic lipoproteins (those with apo B-100) from the circulation into the arterial wall. These lipoproteins bind to the subendothelial matrix, where they may become oxidized or otherwise modified. Both injury and the presence of modified apo B-100-containing lipoproteins stimulate the endothelium to secrete adhesion factors and chemokines. The adhesion factors, such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), arrest mononuclear cells from the bloodstream. The chemokines induce the mononuclear cells to transit the endothelium into the arterial wall, infiltrate the subendothelial space, and differentiate into macrophages. Early plaque formation involves accumulation of these inflammatory cells, which phagocytose cholesterol and other lipid material derived from the modified lipoproteins retained in the subendothelial space, becoming "foam cells." Subsequently, such cells die and discharge lipid material, leading to accumulation of lipid and necrotic debris (atheromatous gruel) so that, as plaques "mature," their central areas become necrotic and calcified.

Plaques may gradually narrow the arterial lumen, disrupting laminar blood flow, and causing stenosis and

distal ischemia. More commonly, however, CVD events are triggered by inflammation leading to plaque rupture, which provokes acute thrombus formation leading to abrupt and dramatic reductions in blood flow, severe downstream ischemia, and even tissue death (infarction). Any organ may be affected, but the most common atherosclerotic syndromes involve arteries supplying the heart (angina pectoris, myocardial infarction [MI], ischemic cardiomyopathy with heart failure), brain (stroke, multi-infarct dementia), kidneys (nephrogenic hypertension, renal failure), and extremities (intermittent claudication, ischemic ulcers, amputations).

To summarize, atherogenesis is a continuum, with substantial overlap of developmental stages. It can be understood as a set of sequential events in which characteristic biological factors exert important influences at each stage of the process. The critical factors are:

- endothelial injury and adhesion factors
- infiltration and subendothelial retention of atherogenic lipoproteins
- infiltration and activation of inflammatory cells and cytokines
- plaque rupture
- thrombosis and coagulation

### Estrogen and etiological factors in cardiovascular disease

The biological plausibility of cardioprotection by MHT is supported by a body of investigations demonstrating favorable effects of estrogen on numerous factors involved in the pathogenesis of atherosclerosis. Paradoxically, the likelihood that estrogens could also have adverse effects on risk of CVD events under certain circumstances is also supported by the same studies.

#### Effects on lipids

The Postmenopausal Estrogen/Progestin Interventions (PEPI) prospective

trial, which compared conjugated equine estrogen (CEE) alone with 3 different CEE-progestin combinations and placebo in 875 healthy, postmenopausal women aged 45 to 64 years, showed an increase in HDL-C and decreases in LDL-C and fibrinogen in women receiving estrogen.<sup>22</sup> Many other studies have also shown favorable lipid effects, including lowering of LDL-C and Lp(a) and raising of HDL-C levels.<sup>23-26</sup> The (potentially) beneficial increase in HDL-C observed with oral administration of estrogens may be attenuated if estrogens are supplied by a non-oral route (ie, transdermally).<sup>25,27</sup>

#### Estrogens, progestins, and endothelial function

Experimental observations demonstrate that estrogen increases NO production in endothelium by inducing NO synthase activity.<sup>28,29</sup> Estrogen-mediated lowering of plasma concentrations of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, may contribute to the latter effect.<sup>30</sup> Estrogens may also improve arterial vasodilation by local inhibition of angiotensin-converting enzyme (ACE)-1<sup>31</sup> and by increasing activity of ACE-2, favoring production of the smooth muscle relaxer, angiotensin 1-7.<sup>32-34</sup> Other effects of estrogen that favor vasodilation include increasing prostacyclins<sup>35</sup> and, possibly, reducing endothelin-1.<sup>36,37</sup>

Although one human study failed to find increased circulating NO levels in estrogen-treated women,<sup>38</sup> numerous studies have shown that estrogen treatment improves EF (measured by FMD) in young ovariectomized<sup>39</sup> and recently menopausal<sup>24,31,40-42</sup> women. In older women with significant existing atherosclerosis the effect of estrogen on FMD is blunted or absent.<sup>43-45</sup>

Other local effects of estrogens on arteries include reduced endothelial expression of the adhesion factors E-selectin, ICAM-1, and VCAM-1, as well

as increased Fas ligand,<sup>46-48</sup> which could contribute to improvement in arterial compliance<sup>49-51</sup> and enhance FMD.<sup>24,52</sup> Estrogen treatments also reduce blood pressure<sup>53-55</sup> and microalbuminuria,<sup>56</sup> a marker of endothelial injury in the renal glomerulus.

Most studies have found that the commonly employed MHT progestin, medroxyprogesterone acetate, antagonizes estrogen's effect on EF in a dose-dependent manner,<sup>36,40,52,57</sup> perhaps in part by increasing endothelin-1.<sup>36</sup> Similar antagonism has been observed with another synthetic progestin, norethisterone.<sup>58</sup> However, there is some evidence that progesterone, the "natural" ovarian progestogen, may antagonize estrogen less or not at all with regard to EF.<sup>41</sup> Progesterone given without estrogen appears to have no adverse effect on vascular function<sup>59</sup> and may even improve<sup>60</sup> FMD in postmenopausal women. Further studies are needed to determine whether some progestogens may have more favorable vascular effects than others.

### Estrogen and inflammatory factors

It is now clear that inflammatory processes are involved in the formation, evolution, and eventual rupture of atherosclerotic plaques.<sup>61-63</sup> Aging and menopause are associated with increases in inflammatory biomarkers, in particular, interleukin-6 (IL-6) and C-reactive protein (CRP).<sup>64</sup> Moreover, elevated levels of one or both of these cytokines have been shown to predict both frailty and chronic illness in the elderly,<sup>65,66</sup> including CVD.<sup>67</sup> As noted above, in the early stages of plaque formation, E-selectin, ICAM-1, and other adhesion molecules attach circulating leukocytes to the vascular wall at sites of endothelial injury. Adherent cells transit the endothelium to enter the vascular intima where they phagocytose lipid particles and transform into foam cells, creating a fatty streak.<sup>68</sup> These cells, having invaded the arterial

intima, amplify the atherosclerotic process by releasing cytokines, including IL-6, interleukin-1 (IL-1), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which attract and activate additional inflammatory cells. Cytokines released from leukocytes and activated platelets also stimulate smooth muscle hyperplasia, a characteristic of the transition from fatty streak to mature plaque. In the final stages of plaque evolution, metalloproteinases that lyse collagen, notably matrix metalloproteinase-9 (MMP-9), are released from infiltrating inflammatory cells, causing plaque rupture, local thrombosis, and vascular occlusion.<sup>69,70</sup> Consistent with this concept, a variety of circulating cytokines have been shown to predict cardiovascular event risk, independent of lipids.<sup>71,72</sup>

**CRP and IL-6.** High CRP is a marker of systemic inflammation that predicts CVD event risk in both men<sup>73-80</sup> and women.<sup>81-83</sup> CRP was the best non-lipid biochemical predictor of subsequent CVD events in the Nurse's Health Study (adjusted relative risk [RR], 1.7; 95% confidence interval [CI], 1.2-2.4 for CRP  $\geq 3.0$  mg/L vs  $<1.0$  mg/L)<sup>82</sup> and in the Women's Health Study (adjusted RR, 4.4; 95% CI, 2.2-8.9 for the highest vs the lowest CRP quartile).<sup>84</sup>

IL-6 is the proximate stimulus for hepatic production of CRP, and in one large prospective study, IL-6—but not CRP—levels independently predicted CVD event incidence. However, in another study, neither CRP nor IL-6 was associated with CVD risk.<sup>85</sup> Some data have suggested that CRP may be a better marker for CVD event risk than for prevalent atherosclerosis,<sup>86</sup> although coronary artery calcification (CAC), measured by CT and given as a CAC score, is correlated with CRP concentrations.<sup>87</sup>

In the Women's Health Initiative (WHI) observational study, occurrence of first MI or death from CVD was associated with significantly higher median baseline levels of CRP and IL-6.<sup>81</sup> MHT use in the WHI interventional trial was

associated with significantly elevated median CRP levels but not IL-6. In other studies in which oral estrogen increased CRP levels, no effect was seen on IL-6,<sup>83,88,89</sup> nor were changes in IL-6 observed during continuous transdermal MHT.<sup>89,90</sup>

In the PEPI trial,<sup>91</sup> oral CEE increased CRP by 85% compared with baseline. In studies comparing oral and transdermal estrogens, oral treatment increased levels of CRP, whereas transdermal estrogen did not,<sup>27,89,92</sup> a finding also seen in a trial of transdermal estradiol (E2) versus placebo.<sup>90</sup> One study of oral E2 combined with cyclic progesterone, however, failed to show a CRP elevation.<sup>46</sup> To summarize, neither oral nor transdermal estrogen appears to affect IL-6, and estrogen's effects on CRP depend on route of administration. Taken together, these findings suggest that the oral estrogen-induced increase in CRP is an "artifact" of its first-pass effect to stimulate hepatic protein synthesis.

**Adhesion factors.** E-selectin promotes binding of inflammatory cells to the vascular endothelium, and E-selectin levels tend to be higher in humans with hypertension, obesity, and diabetes.<sup>93,94</sup> In case-control studies, patients with angiographic evidence of atherosclerosis<sup>95,96</sup> or those hospitalized for MI<sup>97</sup> had significantly elevated E-selectin levels compared with controls; however, in another angiographic study,<sup>98</sup> this was not the case. A prospective study showed that baseline E-selectin levels were higher in subjects who subsequently experienced incident CVD events and were independently associated with degree of atherosclerosis, estimated from carotid intima-media thickness (CIMT; hazard ratio [HR], 2.01; 95% CI, 1.14-3.62 for extreme quartiles).<sup>99</sup> E-selectin has been reported to be increased in postmenopausal women compared with cycling women.<sup>100</sup> Accordingly, both oral<sup>46,91,101,102</sup> and transdermal<sup>48,90</sup> MHT reduced E-selectin levels in prospective studies.

In *in vitro* studies, CRP and TNF- $\alpha$  upregulate soluble ICAM-1 expression in coronary endothelial cells by as much as 10-fold.<sup>103,104</sup> In specimens obtained at carotid endarterectomy, ICAM-1 mRNA and protein are increased in plaques compared with normal endothelium and in high-grade compared with low-grade lesions.<sup>105</sup> Plasma ICAM-1 levels have been found to be elevated in dyslipidemic<sup>106,107</sup> and diabetic<sup>108</sup> patients. Elevated levels of ICAM-1 also were seen in some,<sup>96,109</sup> but not all studies<sup>95</sup> of individuals with angiographic evidence of CVD. Higher ICAM-1 levels have been reported in patients hospitalized for MI compared with controls.<sup>110</sup>

Three prospective studies have reported increased CVD risk associated with elevated ICAM-1 levels.<sup>84,99,103</sup> Oral<sup>46,83,111</sup> and transdermal<sup>48,90</sup> estrogen treatments have been reported to decrease circulating ICAM-1 levels. However, in one prospective trial, oral E2 did not alter ICAM-1 levels.<sup>88</sup>

**Coagulation proteins.** Fibrinogen is a hepatic protein that is converted by activated thrombin to fibrin, the fibrous component of clots. Although involved in coagulation, fibrinogen is also an acute phase reactant, and as a powerful positive marker of inflammation, it appears to be related to atherosclerosis and CVD. An increased incidence of MI or sudden death has been associated with higher concentrations of fibrinogen.<sup>72,80,112</sup>

Plasminogen activator inhibitor-1 (PAI-1), another acute phase reactant involved in the coagulation pathway, has also been shown to be elevated in patients with prevalent CVD compared with healthy controls<sup>113</sup> and to be associated with increased risk of CVD events.<sup>114</sup> In the PEPI trial<sup>22</sup> and other studies<sup>46,115,116</sup> oral estrogen significantly lowered fibrinogen levels and PAI-1, whereas in some prospective trials<sup>117,118</sup> neither oral nor transdermal E2 altered fibrinogen concentrations.

**MMP-9.** MMP-9 belongs to a family of enzymes that degrade collagen. The role of MMPs produced by plaque inflammatory cells in promoting rupture of the fibrous cap is supported by observations in both experimental<sup>119</sup> and human<sup>69,120</sup> atherosclerosis. High levels of local intralésional MMP expression are characteristic of advanced, but not early, plaques.<sup>120</sup> Elevated plasma MMP-9 levels are strongly associated with prevalent symptomatic CVD<sup>121,122</sup> and are predictive of cardiovascular event risk.<sup>69,123</sup>

Although estrogens appear to be potent inducers of several different matrix metalloproteinases in a variety of cell types,<sup>124-126</sup> MHT has been variously reported to have no effect on,<sup>90</sup> to increase significantly,<sup>89,127</sup> or even to decrease<sup>83</sup> circulating levels of MMP-9 in women. The wide disparity in the results of these studies may reflect variations both in production by vascular inflammatory cells and also release of MMP-9 from platelets during sample processing.<sup>128</sup> It may also be due to variability in the severity of atherosclerosis, and hence in the number and activity of inflammatory cells susceptible to stimulation by estrogen.

### Estrogens and clotting/fibrinolysis

Levels or activity of several clotting factors have previously been correlated with adverse CVD events.<sup>85,129-131</sup> Most effects of oral MHT on clotting are thought to be mediated by high estrogen concentrations during "first-pass" through the liver after absorption into the portal circulation. With the notable exception of fibrinogen, most clotting and fibrinolytic factors are affected by oral estrogens or selective estrogen receptor modulators (SERMS) in a direction consistent with an increase in thrombosis.<sup>27,111,115,117,118,132,133</sup> Oral estrogen has been reported to stimulate certain hepatic clotting factors (factors VII, XII, prothrombin), decrease anti-clotting factors (antithrombin-III, PAI-1, tissue plasminogen activator, activated

protein C), and result in greater production of products of fibrinolysis (fibrin split products, D-dimer), consistent with accelerated intravascular thrombus formation. These effects are generally weaker or absent with transdermally administered estrogen.<sup>117,134,135</sup> However, the extent, specific factors affected, and clinical relevance of these effects remain controversial. In fact, some studies have suggested that MHT has no adverse effects, or even beneficial effects, on coagulation through increased activation of fibrinolysis.<sup>92,136-138</sup> In one prospective study, MHT was associated with significant increases in prothrombin fragments 1+2, thrombin-antithrombin (TAT) complex, and fibrin D-dimer at 3 months, but the changes became less pronounced with longer treatment.<sup>139</sup>

### Estrogen as an antioxidant

Oxidation plays an important role in atherogenesis, and estrogens have been shown to be potent antioxidants in a variety of experimental biological systems.<sup>140,141</sup> Transdermal, but not oral, administration of estrogen to postmenopausal women has been found to reduce plasma or urine levels of isoprostane, a marker of oxidative damage to fatty acids found in lipids. Both oral and transdermal estrogens have been shown to reduce LDL-C oxidation and to have other antioxidant effects in lipoprotein and arterial wall components, with likely antiatherogenic effects.<sup>142-145</sup>

### Estrogen's biphasic effects:

#### A summary

To summarize, favorable effects of estrogens on circulating lipoproteins, endothelial function, inflammation, and oxidation may all contribute to protective benefits of MHT in the early stages of atherogenesis. In contrast, adverse effects of estrogens on inflammatory cytokines, MMP-9, and clotting/fibrinolysis, along with the loss of the favorable endothelial response to estrogen in women who are several years post-

menopausal could all help explain why starting MHT years after the menopause might be harmful, especially for women with advanced pre-existing atherosclerotic plaques.

### Risk/benefit ratio for MHT

A well-established benefit of MHT is reduction in risks of osteoporosis-related fractures.<sup>146-148</sup> Other likely benefits include lower risk of colon cancer<sup>123,149</sup> and reduced risk of type 2 diabetes.<sup>150,151</sup> On the negative side, MHT consisting of estrogen plus a progestogen, especially medroxyprogesterone acetate, appears to increase age-adjusted breast cancer incidence by 20% to 30% after just 5 years of treatment.<sup>152-155</sup> The overall question of MHT and breast cancer risk remains complicated, however. For example, women in the estrogen-only arm of the WHI showed a (nonsignificant) trend for lower breast cancer rates compared with placebo-treated women (HR, 0.77).<sup>156</sup> Moreover, in a recent large study of MHT carried out in Europe, the association of estrogen-progestogen combinations with breast cancer risk varied significantly according to the type of progestogen: the RR was 1.00 (0.83-1.22) for estrogen-progesterone, 1.16 (0.94-1.43) for estrogen-dydrogesterone, and 1.69 (1.50-1.91) for estrogen combined with other progestogens. Thus, it seems likely that the presence and type of progestogen used, rather than the estrogen component, determines breast cancer risk.

Oral MHT has also been associated with increased risk of stroke in some studies<sup>123, 156, 157</sup> but not others.<sup>158,159</sup> The risk of stroke appears to be strongly dose-related and may be increased only at doses of CEE 0.625 mg/d or higher.<sup>160</sup> Finally, venous thromboembolic disease is increased when MHT is given orally, but this also appears to be positively related to dose,<sup>123,161</sup> and the risk is diminished or perhaps even absent when the estrogen is administered

transdermally.<sup>162,163</sup>

Thus, calculation of a risk/benefit ratio for MHT is clearly a nuanced problem in which hormonal agents employed, dose, and route of administration all play important roles. Given the fact, however, that CVD is the greatest source of morbidity and mortality in postmenopausal women, it seems inescapable that the overall balance of benefit to risk for long-term MHT depends on whether it is truly cardioprotective in a clinical setting.<sup>164</sup> In other words, in settings where MHT does not reduce CVD risk, this risk/benefit ratio of MHT shifts decisively from positive to negative.<sup>165,166</sup>

Clearly, the striking contrast between the consistent decrease in CVD with MHT in observational studies and the generally adverse effect on CVD reported in most randomized clinical trials raises questions of utmost clinical importance. Naturally, in comparing observational with clinical trial data, the clinical trials have been assumed to provide the more conclusive medical evidence upon which practice should be based. Thus, clinical trial results form the basis of the current official guidelines from the FDA, American Heart Association, and other groups, which state that MHT should be used for the shortest time possible and not for CVD prevention. Critical differences exist, however, between the setting of these clinical trials and that of typical clinical practice. This discrepancy requires a re-evaluation of the issue, which is examined below.

### Clinical studies of MHT and atherosclerosis

Consistent with the above-noted effects of estrogens on cardiovascular risk factors, several large observational studies in a variety of cohorts have shown consistently that MHT use is associated with a decrease in CVD incidence and all-cause mortality of approximately 40% to 50%.<sup>158,160,167-172</sup> Although these

findings strongly suggest that MHT use causes this reduction, nonrandomized, observational studies may be skewed by a variety of biases, most notably the "healthy user bias," in which those women choosing to undertake MHT are generally healthier and at lower risk than women not using hormones. Despite the fact that adjustment for this bias generally did not eliminate the apparent benefit of MHT, the lack of clinical trial data led to organization of several randomized controlled trials in the late 1990s to better define the potential role of MHT in CVD prevention.

Results of these trials, however, were surprisingly negative. The Heart and Estrogen/Progestin Replacement Study (HERS), a large randomized, controlled secondary prevention trial, detected no reduction (and even an early increase) in cardiac events with MHT.<sup>161,173,174</sup> The WHI hormone trials, intended to investigate primary prevention, found a small, statistically nonsignificant increased risk (HR, 1.24; 95% CI, 0.97-1.60) for cardiac events in women treated with estrogen plus progestogen (E+P)<sup>175</sup> and no significant reduction of CVD risk (HR, 0.95; 95% CI, 0.79-1.16) in those treated with E alone.<sup>176</sup>

Why were these trials adverse when observational studies seemed to show benefit? One of the critical differences between these 2 types of studies is the fact that MHT was initiated much later in the HERS and WHI populations than that in usual clinical practice.<sup>177</sup> For example, women in the Nurse's Health Study (NHS), in which strong CVD protection was observed, initiated MHT at a mean age of 51 years,<sup>178</sup> the average age of menopause in the United States. Moreover, NHS participants who started MHT near menopause experienced significant CVD protection (RR, 0.66 for E alone; RR, 0.72 for E+P), whereas those who initiated MHT 10 or more years after menopause, like the participants in the WHI trials, did

not (RR, 0.87 for E alone; RR, 0.90 for E+P).<sup>179</sup> In the WHI, women initiated MHT at an average age of 63 years, approximately 12 years after menopause. However, those women in the WHI who started MHT within 10 years of menopause or before age 60 years, had CVD benefit, while women who started later did not.<sup>123,156,175,180</sup>

Because atherosclerosis often starts in early to middle adulthood and then progresses rapidly after the menopause,<sup>14,181</sup> a significant fraction of the older subset of WHI women likely already harbored subclinical atherosclerosis at randomization, although this was not measured at trial entry. Thus, their treatment was not true “primary prevention” as described in animal models and as conceptualized at the founding of the trial. Outcomes would have been expected to differ between women older than 60 years (with presumably more subclinical atherosclerosis at baseline) compared with women who were younger at trial entry (with presumably little or no subclinical atherosclerosis).

Evidence to support this explanation comes from recent post hoc analyses of the E+P WHI trial that showed a nonsignificant trend toward reduced CVD rates (HR, 0.89) among women less than 10 years postmenopausal at time of starting MHT, whereas excess risk occurred in women more than 20 years postmenopausal (HR, 1.71).<sup>175</sup> Further, in the E-alone trial,<sup>176</sup> the trend toward CVD protection in women aged 50 to 59 years at trial entry was even stronger (HR, 0.63), while risk continued to be increased in women older than 70 years (HR, 1.11). Similar results were found when data from E-alone and E+P groups were combined.<sup>182</sup> Corroborating this point, WHI subjects randomized by age 59 and treated with E-alone were found to have significantly less CAC approximately 2 years after the trial was terminated compared with women given placebo.<sup>183</sup> Thus,

the prevalent assumption that the WHI and HERS trials have proven that MHT invariably has neutral, or even adverse, effects on CVD ignores the fact that clinical MHT use is almost always started at or near the menopause, the time at which MHT appeared favorable in the WHI.

### **KEEPS: Testing the timing of initiation hypothesis**

The ongoing Kronos Early Estrogen Prevention Study (KEEPS) is designed to address the question, “Is early-start MHT beneficial rather than harmful for atherosclerosis?” The KEEPS compares effects of low-dose oral conjugated estrogen vs transdermal estradiol vs placebo on progression of atherosclerosis measured by CIMT by ultrasound and CAC by CT. Importantly, regardless whether the results confirm or deny the hypothesis that early-start MHT is beneficial for atherosclerosis and CVD, KEEPS will provide additional information about the relationship between atherosclerosis imaging and fluid biomarker risk factors for atherosclerosis that promises to be of great value in addressing questions such as: “What are the mechanisms for the discrepancies in effects on CVD risk between the many observational studies and recent randomized clinical trials?” and, assuming that early-start MHT is not uniformly beneficial for atherosclerosis and CVD, “What are clinically useful determinants of the degree of atherosclerosis benefit or risk?”

We expect that heterogeneity in the effects of MHT on measurements of atherosclerosis will be sufficient to allow for examination of mechanisms and predictors of variability. In addition, if some test or combination of tests identified during the KEEPS can be used to identify those recently menopausal women most likely to benefit from cardioprotective effects of MHT, the potential impact on

public health and the savings could be significant. Thus, independent of the outcome of the primary endpoint in KEEPS, it is likely that this study will contribute scientifically and clinically meaningful information.

### **Study design**

KEEPS is an ongoing randomized, blinded, placebo-controlled trial comparing efficacy of 48 months of treatment with 2 different regimens of E+P to reduce progression of atherosclerosis as measured by sequential determinations of CIMT and CAC in recently menopausal women. It thus tests the “timing hypothesis” of Mendelsohn and Karas<sup>14</sup> that MHT reduces CVD if started early in the menopause. The KEEPS has randomized 728 women distributed among 9 US centers to treatment with oral CEE, 0.45 mg, plus a placebo skin patch, 50 mcg of transdermal E2 plus a placebo tablet, or dual placebos. Women treated with active estrogens also receive capsules with 200 mg of micronized progesterone, and women on placebos receive placebo capsules for 12 days/month.

### **Research subjects**

KEEPS participants are healthy female volunteers recruited from the communities surrounding each participating study center. Women must be 42 to 58 years of age, with cessation of menses at age 40 or later; the last menses must have been at least 6 months and no more than 36 months before the time of screening. Subjects may or may not have vasomotor estrogen deficiency symptoms, must not have taken systemic E- or P-containing medication (oral contraceptive or hormone replacement) within 3 months of randomization, and have plasma follicle-stimulating hormone levels measured at greater than or equal to 35 ng/mL and/or plasma E2 levels of less than 40 pg/mL.

Subjects were recruited by advertisements for normal volunteers in print and broadcast news media, posting of flyers at the local hospital and clinics, and at public gathering places such as community centers, by solicitation of referrals from physicians at menopause and women's health clinics at the participating study centers, and/or by mass mailing of recruiting brochures to eligible candidates identified from commercially available mailing lists. At screening and after informed consent, women underwent a complete medical and reproductive history, and physical examination including height, weight, waist and hip circumference measurements, breast examination, pelvic examination, and Pap smear (if not provided from a recent outside exam). At the time of pelvic examination, a vaginal ultrasound

study was obtained to rule out endometrial hyperplasia. Screening procedures included administration of the Beck Depression Scale and the modified Mini Mental State Examination to exclude significant depression and dementia, respectively. Fasting blood was drawn and a urine sample taken for the screening laboratory profile (chemistries, thyroid-stimulating hormone, complete blood count, urinalysis, follicle-stimulating hormone, E2, lipid profile), and a resting electrocardiogram was obtained and evaluated.

Women who qualified according to results of the above examinations and were willing to participate returned for safety imaging studies, which consisted of mammography (if not done in the previous 12 months) and x-ray or electron beam computerized tomography to determine CAC. Because the aim of

the study is to examine effects of MHT in women who do not have significant pre-existing coronary atherosclerosis, women with CAC scores greater than 50 were excluded. Those whose endpoint measurements and safety study outcomes fell within specified acceptable limits were randomized into the study.

Study randomization began in August 2005 and was completed in June 2008, so that all women followed will have completed at least 48 months in the study by June 2012. Attrition rates to date suggest that nearly all of the loss of subjects occurs in the first 12 months, with relatively low rates thereafter. Thus, investigators anticipate that KEEPS will meet or exceed the original study goal of 450 women finishing 48 months of protocol treatment. The last patient visit is scheduled for June 2012 and results should be available soon thereafter. ■

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# Cardio-deleterious implications of declining premenopausal ovarian reserve

► Lubna Pal, MBBS, MRCOG, MS, and Susan M. Hailpern, DrPH, MS

Marking the finality of reproductive senescence, menopause heralds a melee of health-related consequences. Cardiovascular disease (CVD) is the major contributor to postmenopausal morbidity and mortality.<sup>1</sup> A decline in ovarian function, and more specifically declining ovarian estrogen, is appreciated as a critical pathophysiological mechanism in the context of CVD in aging females.<sup>2,3</sup> Despite ongoing debate regarding a possible cardioprotective role of postmenopausal estrogen therapy,<sup>2,4</sup> the beneficial influences of the “estrogen replete” status of premenopausal years on cardiovascular physiology are well substantiated, as is the risk for premature onset of CVD in the context of early menopause. Early (prior to 45 years of age) and premature (prior to 40 years of age) menopause are associated with increased cardiovascular disease, as well as increased all cause mortality and morbidity.<sup>5</sup>

The cardio-protective role of endogenous estrogen is well accepted. However, studies showing no protection or even detrimental effects of postmenopausal exogenous estrogen exposure have caused much confusion.<sup>4</sup> Recent and ongoing studies add credence to a “critical window” hypothesis for the cardiovascular benefits of exogenous estrogen, ie, if initiated prior to

significant atherosclerosis, estrogen is likely to confer cardioprotection<sup>6</sup>; this tenet, however, is far from substantiated. Little attention has been paid to the equally critical concept that the onset of cardiodeleterious processes may begin with declining ovarian function.

## Continuum of decline in reproductive physiology

Although cessation of menses represents a landmark within the paradigm of aging, a continuum of decline in reproductive physiology predates the final menses by decades. Indeed declining reproductive performance, menstrual irregularities, and vasomotor symptomatology predate the final menses by years.<sup>7</sup> Thus, menopause is but one overt milestone in the progression of reproductive aging. A burgeoning body of literature from the field of assisted reproductive technology provides tangible evidence of deteriorating ovarian physiology in the premenopausal years. The concept of “ovarian reserve” reflects the quantity (as well as

the quality) of oocytes and granulosa cell units remaining in the ovaries at any age. Ovarian reserve can be assessed by common biochemical and morphometric parameters.<sup>8</sup> A decline in ovarian reserve with aging is well substantiated and establishes the process of reproductive senescence as a continuum. Of note, differentials exist in the trajectories of age-related decline in ovarian reserve amongst individuals and in populations. Additionally, an increasing number of extraneous influences that may accelerate the aging process are being identified. Elevated levels of the pituitary gonadotropin follicle stimulating hormone (FSH) have long been recognized as accompaniments of reproductive aging.<sup>8</sup> A direct and positive correlation between basal FSH and aging is well established, and a rise in FSH in aging women predates the ovulatory deficiencies and menstrual disturbances that characterize the menopausal transition.<sup>7,8</sup>

## Multisystem implications of reproductive aging

Accruing, yet sparse, data provide evidence of the multisystem ramifications of declining ovarian reserve in the premenopausal years. An increased likelihood of early menopause—and risk of associated morbidities—is described in premenopausal women with reduced ovarian reserve.<sup>9,10</sup> In a cross-sectional

Lubna Pal, MBBS, MRCOG, MS

Department of Obstetrics & Gynecology and Women's Health (Reproductive Endocrinology and Infertility)  
Albert Einstein College of Medicine  
Bronx, New York

Susan M. Hailpern, DrPH, MS

Associate Director of Outcomes Research  
LipoScience, Inc.  
Raleigh, North Carolina

FIGURE

### Menopause terminology: STRAW staging system

		Final menstrual period (FMP)						
Stages	-5	-4	-3	-2	-1	0	+1	+2
Terminology	Reproductive			Menopausal transition		Postmenopause		
	Early	Peak	Late	Early	Late*	Early*		
Duration of stage	Variable			Variable		Perimenopause		
						Ⓐ 1 yr	Ⓑ 4 yr	Until demise
Menstrual cycles	Variable to regular	Regular		Variable cycle length (>7 days different from normal)	≥2 skipped cycles and an interval of amenorrhea (≥60 days)	Amen × 12 mo		
Endocrine	Normal FSH		↑ FSH	↑ FSH		↑ FSH		

\*Stages most likely to be characterized by vasomotor symptoms. ↑ = elevated.

Adapted with permission from Soules MR, et al. Executive summary: Stages of Reproductive Aging Workshop (STRAW). Fertil Steril. 2001;76:874-878.

study of regularly menstruating premenopausal and healthy, albeit infertile women, Chu et al demonstrated a worsening lipid profile (increasing total cholesterol and decreasing high-density lipoprotein [HDL] levels) in association with declining ovarian reserve, as reflected by increasing FSH levels.<sup>11</sup> Worsening renal function<sup>12</sup> and enhanced likelihood for pregnancy-induced hypertension<sup>13</sup> represent additional examples of CVD-related risks associated with declining ovarian reserve. Adverse skeletal effects include enhanced bone turnover and higher prevalence of low bone mineral density (BMD) in regularly menstruating premenopausal women with reduced ovarian reserve.<sup>14</sup> Recent data demonstrate direct stimulatory effects of FSH on osteoclasts, explaining at least one mechanism through which declining ovarian reserve may accelerate bone loss.<sup>15</sup> Other potential mechanisms include declining estrogen and androgen levels in the context of declining ovarian reserve.<sup>14,16</sup>

To better understand the cardiodeleterious implications of declining ovarian reserve in premenopausal years, we undertook targeted analyses of the publicly accessible NHANES data from 1999 to 2002.<sup>17</sup> Serum FSH levels were available for women aged 35 to 60 years of age. Analyses were conducted among non-pregnant, premenopausal participants women aged 35 to 50 years; premenopausal status was established based on a last menstrual period within 12 months (n = 581). Associations between serum FSH levels and surrogate

markers for CVD (systolic and diastolic blood pressure [BP], fasting lipid profile, serum homocysteine, and insulin levels) were assessed. Results from unadjusted analyses were consistent with multivariable weighted analyses conducted to account for survey design (adjusted for age, race, BMI, smoking, exercise, menstrual regularity and socioeconomic status [SES]). Women with FSH levels of 10 to 19 mIU/mL demonstrated a nearly 3 mm Hg increase in DBP ( $P = 0.031$ ) and a 4 mm Hg increase in SBP ( $P = 0.072$ ) compared with women whose FSH was less than 10 mIU/mL. Each log unit increase in FSH was accompanied by deterioration in biomarkers recognized as surrogates for CVD including increasing serum insulin ( $P = 0.002$ ) and homocysteine ( $P = 0.004$ ) levels. Premenopausal women with FSH 20 mIU/ml or higher were significantly more likely to have higher homocysteine levels ( $P = 0.005$ ) compared with women whose FSH was less than 20 mIU/ml on adjusted analyses. These analyses provide evidence that declining ovarian reserve in premenopausal years is accompanied not only by worsening surrogates for CVD risk, but also with a rise in BP, a clinical end point in itself. These observed relationships appear to be independent of previously recognized determinants of CVD, such as age, race, body mass index, menstrual regularity, smoking status, exercise, and SES, and they suggest that initiation of processes culminating in CVD predate the final menses by decades.

## Conclusion

Collectively, multisystem ramifications of declining ovarian reserve in the premenopausal years have been identified. Skeletal attrition and processes recognized as surrogates for CVD are well under way in the premenopausal years, concomitant with deteriorating ovarian reserve. It is of interest that the 2 organ systems identified as being adversely influenced by deteriorating ovarian reserve, ie, skeletal and cardiovascular, are also the ones that contribute to the bulk of health burden in aging populations. Given this observation, any absolute categorization of reproductive decline, for example into premenopausal, perimenopausal, and postmenopausal periods, may completely miss a "causality" to specific pathophysiological relationships that may underlie initiation of diseases under consideration. If the goal of preventive strategies is to curtail pathophysiological processes at their inception, it is necessary to recognize that cardiodeleterious processes are initiated concomitant with declining reproductive physiology in years predating the final menses. If substantiated, these concepts will allow for timely intervention with targeted preventive strategies that have the potential to translate into reduced health burden attributable to CVD and skeletal fragility and possible savings in health care dollars. ■

## Disclosures:

Drs Pal and Hailpern have no conflicts of interest to disclose.

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