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Women's Health Initiative Hormone Therapy Trials: New insights on Cardiovascular Disease from Additional Years of Follow up

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Abstract

Debate and controversy surrounding the benefits and risks of menopausal hormone therapy (MHT) for prevention of cardiovascular disease has continued in the decade since the cessation of the Women's Health Initiative (WHI) hormone therapy interventions. As a result, many women and their physicians have been reluctant to turn to MHT for relief of vasomotor and other menopausal symptoms. However, several follow-up studies of WHI participants provide additional insight into clinical characteristics of women who are more likely to have favorable outcomes and lower rates of adverse events associated with MHT. This report focuses on those studies that identify characteristics and biomarkers helpful in stratifying risk for an individual. Incorporation of these factors into a benefit:risk model could assist in patient-oriented decision making regarding use of MHT. Personalizing treatment offers the potential to minimize risk and improve health outcomes.

Keywords

Conjugated equine estrogens; Medroxyprogesterone acetate; Myocardial infarction; Stroke; Timing hypothesis

Introduction

Cardiovascular disease remains the leading cause of death in women [1]. Over the past decade, debate and controversy has raged regarding the benefits and risks of menopausal hormone therapy (MHT) for prevention of cardiovascular disease when results of the Women's Health Initiative (WHI) seemed at odds with observational and epidemiological data indicating that MHT provided primary prevention of coronary heart disease and reduced cardiovascular mortality [2–7]. Since the cessation of the randomized component of the WHI, participants have been followed over the course of three to eight years post-intervention. In addition, secondary analyses in WHI have provided insight into phenotypic characteristics and biomarkers that might be useful in guiding a woman's decision regarding whether to use MHT for management of menopausal symptoms, while minimizing risk for adverse cardiovascular events [8]. This report summarizes these and other relevant studies.

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Conflict of Interest:
Virginia M. Miller declares that she has no conflict of interest.
JoAnn E. Manson declares that she has no conflict of interest.

The WHI: Design and Cardiovascular Outcomes

In order to put the follow-up studies into context, a brief review of the WHI study design and outcomes is warranted. The WHI hormone trials included healthy postmenopausal women aged 50–79 (mean age 63 years) [9]. Older women are at greater risk for coronary heart disease (myocardial infarction and coronary death), the main outcome of interest, and thus were targeted for enrollment. Women with a uterus were randomized to daily oral conjugated equine estrogens (CEE; 0.625 mg) plus medroxyprogesterone acetate (MPA, 2.5 mg) or placebo. Women who had undergone a hysterectomy (41% also had oophorectomy) were randomized to daily oral CEE or placebo. The CEE+MPA trial was terminated after 5.6 years because of increased risk of myocardial infarction, stroke, venous thromboembolism and breast cancer. The CEE trial was terminated after 6.8 years of treatment because of increased risk of stroke. Continued follow-up during the post-intervention phase of both trials indicated that cardiovascular risks returned to baseline, or became substantially attenuated, following cessation of treatment [10•, 11].

Biological Characteristics: Can Risk be Stratified?

Chronological and Menopausal Age

A major criticism of the design of the WHI was that the average age of the women was about 10 years past the time that most women would initiate use of MHT for relief of menopausal symptoms. However, in defense of WHI's decision to include postmenopausal women across a broad age range, it should be noted that previous observational studies had not indicated clear differences in the relationship between MHT and cardiovascular events according to age group and, at the time the WHI trial was designed, older women were increasingly being prescribed MHT for presumed cardioprotection [9]. Data from basic science studies indicated that MHT initiated close to the time of estrogen-depletion reduced progression of atherosclerotic lesions whereas delaying treatment was without effect [12]. These findings formed the basis for what is known as the "timing hypothesis" or defined a "window of opportunity" for which MHT might provide cardiovascular benefit, the premise for the design of the Kronos Early Estrogen Prevention Study (KEEPS) and the Early versus Late Intervention Trial with Estradiol (ELITE) [13, 14].

Although full results of the KEEPS and ELITE have yet to be published, secondary and follow-up analysis of the WHI data suggest that time past menopause and chronological age influence cardiovascular outcomes on MHT. In pooled data from both the CEE+MPA and CEE trials, relative risk for myocardial infarction increased with time past menopause from 0.76 (95% CI 0.50–1.16) for women <10 years past menopause compared to 1.28 (95% CI 1.03–1.58) for women ≥ 20 years past menopause (p for trend = 0.02) [15]. In the CEE alone trial, the hazard ratios for a combined endpoint of coronary heart disease events and coronary revascularizations were significantly lower for women assigned CEE than placebo among those aged 50–59 years, but not for those 60–69 or 70–79 years [16]. Coronary arterial calcification, measured at the end of the CEE alone trial, was also significantly lower for women assigned CEE, compared to placebo, among women in the 50–59 year age group [17].

Analysis of the CEE group over 11 cumulative years of follow up (including the intervention period and a median of 5.9 years of post-stopping follow-up) also indicated a lower incidence of coronary heart disease, myocardial infarction, and all-cause mortality in women who were randomized to CEE at 50–59 years of age, but no reductions in these outcomes were observed in older women (Table 1) [10•]. These results are consistent with epidemiological evidence showing that initiation of estrogenic treatment in close proximity

to estrogen-depletion resulting from oophorectomy is associated with reduced all-cause and cardiovascular mortality [18].

Similar modifying effects of chronological age and time past menopause were not found for stroke risk either in the primary analysis or follow-up studies [4, 10•]. Indeed, the 'window of opportunity" for stroke prevention may not be comparable to that for cardiac events [19]. Additional investigations are needed to better understand, determine and differentiate clinical characteristics and risk factors for cerebrovascular compared to coronary vascular disease.

Biomarkers

Identification of a panel of new biomarkers beyond the traditional cardiovascular risk factors of age, elevated total or low-density lipoprotein cholesterol (LDL-C), low high density lipoprotein cholesterol (HDL-C), smoking and hypertension, (D'Agostino 2008) that would help to stratify cardiovascular risk for women choosing to use MHT for menopausal symptoms has been generally disappointing to date. However, lipid biomarkers and the constellation of risk factors associated with the metabolic syndrome may have utility in risk stratification in postmenopausal women. In a case control biomarker study within the combined CEE+MPA and CEE trials of the WHI, women with an LDL/HDL ratio > 2.5 had about a 73% higher risk for a CHD event if assigned to MHT compared to placebo, while women with favorable lipid status had a suggestion of lower CHD risk on MHT (Table 2) [20]. One possible explanation for the high predictive value of the LDL-cholesterol level and LDL/HDL ratio may lie in the relationship between LDL-cholesterol and stage of atherosclerosis, as well as with thoracic fat. In examination of correlations between intra-hepatic and intra-thoracic fat deposition with cardiovascular risk factors among women being screened for KEEPS, LDL-cholesterol and triglyceride levels were associated with thoracic fat even after adjustments for body mass index. Moreover, thoracic fat as evaluated from computed tomography of the coronary arteries was associated with coronary arterial calcification [21••].

Another biomarker associated with LDL-cholesterol is 27-hydroxycholesterol, which can act as an estrogen receptor antagonist and thus may contribute to risk prediction. However, in a nested case-control study of 350 cases of coronary heart disease (and 813 controls without cardiovascular disease) in the WHI, this biomarker did not independently identify women at risk of coronary events on MHT [22].

Various cardiometabolic parameters associated with metabolic syndrome represent a high risk phenotype for development of cardiovascular disease and may have utility in stratifying cardiovascular risk for women contemplating use of MHT. In the WHI, women meeting criteria for metabolic syndrome at baseline had increased risk for adverse cardiovascular events with MHT [23••]. Specifically, women with the constellation of variables for metabolic syndrome had an elevated risk of coronary heart disease on MHT compared to placebo (HR=2.26 for women with metabolic syndrome and 0.97 for women without this condition; p-value for interaction = 0.032; Table 2) while the individual components of the syndrome were not significant modifiers of MHT effect [23••]. Interactions among lipid and glucose metabolism and hypertension may synergistically affect various cellular mechanisms of disease processes. For example, although individual components of the metabolic syndrome affect specific platelet functions, interactions among vascular elements as measured by types of cell-membrane derived microvesicles (see below) are associated with carotid intima medial thickening [24].

Analysis of inflammatory cytokines or proteins to identify cardiovascular risk on MHT has been disappointing. Indeed, investigation of a variety of proteins and cytokines associated

with inflammation, coagulation and vascular matrix remodeling including high sensitivity C-reactive protein, interleukin-6, D-dimer, factor VIII, von Willebrand factor or matrix metalloproteinase 6 did not significantly identify risk for coronary heart disease events with MHT [20, 25]. Assessment of Factor V Leiden, however, was useful for identifying women at high risk of venous thromboembolism while on MHT (see below). Identification of biomarkers for risk stratification is limited by the fact that many of the factors are measured at only one point in time. Since, there are either positive or negative feedback interactions among pathways regulating cytokine production to maintain homeostasis, monitoring cytokines at multiple time points might unmask temporal relationships among them that could improve understanding of their contribution to and use in risk stratification.

The relationship of adipokines (beyond the markers of insulin resistance included in metabolic syndrome, as discussed above) to cardiovascular risk with MHT is underexplored. Studies may be limited by assay sensitivity and the more complex relationships of these factors to other cardiovascular risk factors. For example, retinol-binding protein 4, released by the liver and adipose tissue, down regulates glucose transporters and is implicated in obesity, type 2 diabetes and metabolic syndrome. Although weakly associated with triglycerides in newly menopausal women being screened for the KEEPS trial, there was a curvilinear relationship of the binding protein with coronary calcium scores, with women at both lower and higher quartiles having an association with coronary calcium [26]. New insights into the complex relationships among fat depots, insulin insensitivity and cardiovascular risk with MHT may be gained from continued analysis of samples from participants in WHI, KEEPS and other studies.

One emerging potential set of biomarkers that may help to identify ongoing disease processes is cell-membrane derived microvesicles (also referred to as microparticles) [27]. Upon activation, vascular endothelial cells, leukocytes and platelets shed sealed vesicles less than one micron in diameter containing bioactive material. Validated methodologies are available to quantify and identify cellular origins of microvesicles as well as their thrombogenic potential [28••]. Numbers of thrombogenic microvesicles increase with decreases in endogenous estrogen suggesting that activation of the vascular compartment signaling early disease processes occur in the early peri-menopausal period [29]. For example, in women being screened for participation in KEEPS, numbers of endothelium-derived microvesicles correlated with coronary arterial calcium scores > 100 Agatston units even in women in whom other cardiovascular risk parameters (lipids, blood pressure, fasting blood glucose and triglycerides) were within normative ranges [30]. Thus, analysis of microvesicles may identify coronary disease processes and perhaps cardiovascular risk prior to development of clinical symptoms. A nested case-control study of evaluation of microvesicles at baseline in women of the WHI has not yet been conducted but could test this hypothesis.

In addition, cellular origin of microvesicles may be key in differentiating early disease processes in carotid compared to coronary arteries. For example, in women being screened for KEEPS, platelet-derived and thrombogenic microvesicles were associated with carotid intima medial thickness, whereas endothelium-derived microvesicles were elevated with coronary arterial calcification [24, 30].

Genotype

Given the well-known risk for thrombosis in carriers of Factor V Leiden, it is not surprising that in the WHI, Factor V Leiden was predictive of pulmonary embolism and other venous thromboembolic events with MHT. Polymorphisms in the gene for glycoprotein IIIa leu33pro predicted risk for coronary events [25, 31]. With MHT use, it might be expected that polymorphisms in estrogen receptors might affect overall efficacy of treatment and

could contribute to risk for adverse cardiovascular events. In the WHI, estrogen receptor polymorphisms were associated with reduced effects of MHT on plasmin-antiplasmin but not cardiovascular events [32].

In a targeted gene study to evaluate effects of MHT on cardiovascular disease as a complex trait, specific polymorphisms in genes associated with inflammation and not coagulation or fibrinolysis were found to be associated with the presence of coronary arterial calcification and carotid intima-medial thickness in newly menopausal women of KEEPS (Table 3) [33]. Whether these polymorphisms remain associated with disease processes in women using MHT remains to be evaluated. Other gene variants associated with estrogen metabolism and signaling also remain to be evaluated. Another approach to understanding genetic influences of MHT on cardiovascular risk is to evaluate methylation of DNA and how this process is related to endogenous estrogen (and other hormonal levels), time past menopause and current or past use of MHT.

Hormonal Formulations

It is important to keep in mind that only one dose and formulation of estrogen (CEE), with and without a synthetic progestogen (MPA), was used in the WHI. The main estrogenic component of CEE is estrone sulfate and the oral formulation is metabolized further in the liver. Metabolism of oral 17β estradiol is different from that of CEE and would not be expected to yield comparable circulating levels of sex steroid hormones. Moreover, genetic polymorphisms affecting estrogen metabolism will influence the concentrations of hormones in individual women [34–39]. Furthermore, oral and transdermal routes of estrogen delivery may have different effects on thromboembolic risk, with avoidance of first pass hepatic metabolism and emerging evidence for lower thrombotic risk with the latter [40–44].

Since the WHI, many professional societies have recommended use of lower doses of estrogen [45]. Effects of lower doses and different formulations of these products on progression and risk for cardiovascular disease remain to be evaluated. In addition, some of the adverse effects of MHT in the WHI may have been associated with the progestogen, MPA, because of the lower number of adverse cardiovascular events (and breast cancers) observed between the CEE alone and CEE+MPA trials. Evaluation of surrogate and clinical outcomes from randomized trials using lower doses and alternative formulations of estrogen products and other progestogens including natural progesterone will provide important evidence to inform future decision making regarding MHT use.

Conclusions

Results of the WHI have fueled a decade-long debate over the cardiovascular benefits and risks of MHT. Several new observations have emerged from follow-up studies of WHI directed at stratifying cardiovascular risk in menopausal women. Several lessons have been learned from this research including that women with lower baseline risk of cardiovascular events, as evidenced by closer proximity to menopause, younger age, more favorable lipid profiles, and the absence of a metabolic syndrome phenotype, tend to have lower risks of coronary events on MHT than women at higher baseline risk. Also, effects on cardiovascular outcomes have been more favorable for CEE alone than for CEE+MPA, especially for women below age 60. Although consideration of clinical and biomarker variables may assist in risk stratification and individualized decision making regarding MHT, additional research is needed to refine this process. Further research is also needed to differentiate factors contributing to risk for stroke and venous thromboembolism (as well as cancer and other non-CVD outcomes) while on MHT from those factors contributing to risk for coronary

heart disease, as well as to elucidate potential differences in outcomes with other MHT formulations, doses, and delivery systems.

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•• Of major importance

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Table 1

Cumulative annualized incidence rates for clinical outcomes in the WHI CEE-alone trial by 10 year age stratification at enrollment.[a]

	No. (%) of Eventsevents			
Event by Age age Groupgroup, y	**CEE**	**Placebo**	**HR (95% CI)**	***P* Value value for Interactioninteraction**
CHD				
50–59	33 (0.18)	56 (0.31)	0.59 (0.38–0.90)	
60–69	161 (0.65)	168 (.065)	1.00 (0.80–1.24)	.05
70–79	125 (1.01)	121 (0.95)	1.06 (0.82–1.36)	
Total MI				
50–59	27 (0.15)	50 (0.27)	0.54 (0.34–0.86)	
60–69	126 (0.51)	124 (0.48)	1.05 (0.82–1.35)	.007
70–79	101 (0.82)	84 (0.66)	1.23 (0.92–1.65)	
Stroke				
50–59	29 (0.16)	28 (0.15)	1.09 (0.65–1.83)	
60–69	114 (0.46)	94 (0.36)	1.27 (0.97–1.67)	.91
70–79	92 (0.74)	84 (0.66)	1.13 (0.84–1.53)	
Death (all causes)				
50–59	65 (0.35)	89 (0.48)	0.73 (0.53–1.00)	
60–69	254 (1.00)	253 (0.96)	1.04 (0.88–1.24)	.04
70–79	258 (2.02)	239 (1.83)	1.12 (0.94–1.33)	

[a]Modified from Figure Fig. 5 of reference [10•]. The rates were estimated for the overall follow-up period (intervention mean 7.1, follow-up median 5.9 years). P values are for interaction by age.

Abbreviations: CEE, conjugated equine estrogen; CHD, coronary heart disease; WHI, Women's Health Initiative.

Table 2

Cardiovascular risk associated with use of CEE with and without MPA in women of the WHI[a]

	Combined trials			
	Placebo	Hormone Treatmenttreatment	OR (95% CI) for Treatment treatment Effecteffect	*P* Valuevalue
	Case/Control control	Case/Controlcontrol		
Lipids				
LDL cholesterol (mg/dl)				
< 130	31/123	22/129	0.66 (0.34–1.27)	0.03
≥ 130	80/219	122/222	1.46 (1.02–2.10)	
LDL/HDL ratio				
<2.5	38/144	29/165	0.60 (0.34–1.06)	0.002
≥ 2.5	73/198	115/186	1.73 (1.18–2.53)	
Inflammation				
hs-CRP (mg/dl)				
<2.0	46/158	52/178	1.01 (0.63–1.62)	0.16
≥ 2.0	64/179	101/169	1.58 (1.05–2.39)	
Metabolic syndrome meeting three or more criteria in APTIII definition				
No	40/158	47/188	0.97(0.58–1.61)	0.032
Yes	29/100	50/74	2.26 (1.26–4.07)	

[a]Derived from Tables 3 and 2 of references [20, 23••], respectively. Baseline risk assessment followed the APTIII definition of metabolic syndrome and this analysis excluded women with a history of cardiovascular disease, hypertension or diabetes.

Abbreviations: CEE, conjugated equine estrogen; HDL, high density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive Protein; LDL, low density lipoprotein; MPA, medroxyprogesterone acetate; WHI, Women's Health Initiative

Table 3

Description of significant SNPs associated with coronary arterial calcification and carotid intima medial thickening in newly menopausal women being screened for KEEPS[a].

Coronary arterial calcification							
SNP	**Gene Namename**	**Chr.**	**Position (base pair)**	**Risk Alleleallele**	**N**	**Odds Ratioratio**	**P***
rs11465886	IRAK2	3	10225783	G	599	3.909	0.000110
rs17751769	SERPINA1	14	93926410	A	575	1.955	0.000242
rs630014	ABO	9	135139543	A	599	0.508	0.000251
Carotid intima medial thickening							
SNP	**Gene Namename**	**Chr.**	**Position (base pair)**	**Risk Alleleallele**	**N**	**Beta**	**P***
[b] rs2236935	MAP4K4	2	101810474	G	606	0.03697	2.36E-06
rs4796119	CCL5	17	31217201	G	607	−0.0427	3.59E-05
rs739718	IL5	5	131900972	G	607	0.05122	5.02E-05
rs2291299	CCL5	17	31215519	G	607	−0.03179	5.59E-05

*Uncorrected P

[a]Derived from Tables 4 and 5 of reference [33]. Associations for coronary arterial calcification were corrected for waist circumference; association for carotid intima medial thickening were corrected for percent European ancestry, age and pulse pressure.

[b]Associations remained significant after correcting for multiple testing.

Abbreviations: KEEPS, Kronos Early Estrogen Prevention Study; SNP, single nucleotide polymorphism