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Changes in remnant and high-density lipoproteins associated with hormone therapy and progression of coronary artery disease in postmenopausal women

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Abstract

Objective—This study examined the effect of hormone therapy (HT) on the plasma concentration of remnant lipoprotein cholesterol (RLP-C) and high density lipoprotein (HDL) subpopulations and the contribution of HT-related changes in these lipoproteins to the progression of coronary heart disease (CHD) in postmenopausal women.

Methods—Study participants were 256 women who completed the Estrogen Replacement and Atherosclerosis (ERA) trial, a placebo-controlled, randomized trial that examined the effects of 3.2 years of conjugated equine estrogen (CEE, 0.625 mg/day) or CEE (0.625 mg/day) plus medroxyprogesterone acetate (MPA, 2.5 mg/day) on post-menopausal women with established coronary atherosclerosis. Quantitative coronary angiography and plasma RLP-C and HDL subpopulations were assessed at baseline and at follow-up.

Results—Relative to placebo, both CEE and CEE+MPA caused a significant reduction in plasma RLP-C concentrations and a significant increase in $\alpha 1$ and $\alpha 2$ HDL subpopulations. However, in the HT-treated subjects, faster progression of coronary atherosclerosis was observed in women who experienced the greatest reductions in RLP-C and in pre $\beta 1$ HDL subpopulations.

Conclusions—Our data suggest that individual variability in RLP-C and HDL subpopulation response to HT is a predictor of CHD progression. Lipoprotein response to HT may be an indirect marker of susceptibility to other harmful effect of HT in postmenopausal women with established CHD or an indication of formation of dysfunctional lipoproteins.

Keywords

Lipoproteins; hormone therapy; coronary heart disease; angiography

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INTRODUCTION

Randomized controlled trials conducted to assess the effect of hormone therapy (HT) on coronary heart disease (CHD) risk in postmenopausal women have concluded that HT does not reduce the overall risk of CHD (1–6). The lack of protection from CHD occurs in the context of HT-induced beneficial changes in intermediate markers of CHD risk, such as reductions in plasma low-density lipoprotein cholesterol (LDL-C) concentrations and increases in plasma high-density lipoprotein cholesterol (HDL-C) concentrations. The results of these randomized trials are in contrast with those of several previous observational studies and with those of studies conducted in animal models of atherosclerosis, which had suggested a protective role of HT (7,8). It has recently been suggested that the discrepancy in findings between the randomized and observational studies may in part be reconciled by the observation that HT-related CHD risk tends to be lower in women who initiate HT close to menopause and higher in women who initiate HT distant from menopause (9), in conjunction with the fact that HT is usually initiated several years after menopause in the randomized trials but at the time of menopause in the observational studies. The age-dependent effect of HT on CHD risk may be related to the dual effects of estrogen: beneficial, such as reductions in plasma LDL-C and increases in HDL-C concentrations, and harmful, such as increased thrombogenicity, inflammation, and plaque instability (10–12). It has been hypothesized that, in women with CHD, the diseased endothelium may tilt the balance toward the harmful effects of HT (13).

Lipoprotein subfractions may be better indicators of CHD risk than triglyceride, LDL-C and HDL-C concentrations (14–16). We tested the hypothesis that variability in lipoprotein remnant and HDL subpopulation response to HT may play a role in the individual cardiovascular response to HT treatment.

METHODS

Subjects and study design

Subjects were women participating in the ERA trial, a placebo-controlled, randomized study (2,17). Women were enrolled into the study if they were postmenopausal and had coronary artery disease, defined as $\geq 30\%$ stenosis of at least one epicardial coronary artery by quantitative coronary angiography. Women with a history of uncontrolled diabetes or hypertension, deep-vein thrombosis or pulmonary embolism, kidney disease, symptomatic gallstones, or TG levels >400 mg/dL were excluded. A total of 309 women were enrolled in the ERA trial and were randomized to three parallel treatment arms: placebo, conjugated equine estrogen (CEE 0.625 mg/day, as Premarin, Wyeth-Ayerst), and CEE and medroxyprogesterone acetate (CEE 0.626 mg/day and MPA 2.5 mg/day, as Prempro, Wyeth-Ayerst). Paired coronary angiography assessments and plasma samples at baseline and at follow-up were available in 256 women (placebo: N=88; CEE: N=84; CEE+MPA: N=84).

Assessment of plasma lipids, remnant lipoproteins and HDL subpopulations

Fasting blood samples were obtained from study participants at the baseline visit and at the year-1 visit. Blood was drawn in tubes containing 0.1% EDTA and plasma was separated by centrifugation at $1000 \times g$ for 30 min at 4 °C. Plasma total cholesterol (TC) and triglyceride (TG) concentrations were measured by automated assays (18). HDL-C levels were measured after precipitation of apo B-containing lipoproteins with heparin-manganese (19). Apo A-I in plasma and apo C-III in plasma and in HDL were measured with immunoturbidimetric assays (Wako Diagnostics, Richmond, VA) (20).

Plasma concentrations of remnant-like lipoprotein cholesterol (RLP-C) were measured using an immunoseparation technique (Polymedco, Cortlandt Manor, NY) (21).

Apo A-I-containing HDL subpopulations were measured by 2-dimensional gel electrophoresis, as previously described (22). This methodology allows for the separation of HDL into 8 subpopulations (pre β 1–2, α 1–3, pre α 1–3), according to charge, size, and composition. The concentration of each HDL subpopulation was calculated by multiplying its percentage with total plasma apo A-I concentration and expressed as mg/dL of apo A-I.

Coronary angiography

Quantitative coronary angiography was performed at baseline and after approximately 3.2 y of follow-up, as previously described (2,17). Analysis of angiograms was performed in pairs with a previously validated system of cine-projection (SME 3500, Sony, Park Ridge, NJ). The mean intra-operator difference between blinded duplicate measurements of minimal diameter was 0.02 mm. The reference, minimal (the point of greatest narrowing), and average luminal diameters were obtained for 10 proximal epicardial coronary artery segments, as previously described (2,17).

Statistical analyses

The SAS statistical package (version 9.1) was used for statistical analyses. All analyses were based on intent-to-treat. Skewed variables were log-transformed before analysis. Changes in plasma lipid levels, apolipoprotein levels, and mean minimum coronary artery diameter (MMD) were calculated as: Value_{follow-up} – Value_{baseline}. The effects of treatment on plasma concentration of lipids, apolipoproteins, and lipoprotein subspecies were tested by ANOVA. Mixed-model analysis of covariance was used to test the hypothesis of an association between changes in lipoprotein subspecies and changes in MMD. The model was adjusted for baseline minimal coronary lumen diameter, location of the segment in the coronary artery tree, age, body mass index (BMI), race, smoking, hypertension, diabetes, use of lipid-lowering medications, and prior percutaneous transluminal coronary angioplasty. Analyses of MMD change by tertiles of changes in RLP-C and HDL subpopulations were also carried out. These analyses were adjusted for the same CHD risk factors as listed in the mixed-model analysis. A P value <0.05 was set as statistically significant.

RESULTS

Women randomized to placebo, estrogen alone, or the combination estrogen plus progestin were comparable with respect to baseline characteristics (Table 1). Moreover, there were no differences in plasma concentrations of RLP-C, apo C-III, apo A-I, HDL-C, or HDL subpopulations in the three groups of women before randomization, with the only exception of a small difference in HDL pre α 1 particle levels (Table 2).

Over the course of the trial, no significant changes in plasma TG, apo C-III, or RLP-C levels were observed in the placebo arm of the study (Figure 1). Relative to placebo, active treatment with estrogen alone or with estrogen plus progestin was associated with significant overall reductions in plasma RLP-C concentrations (mean change: –2.3 mg/dL, range: –23 to +25 mg/dL; and mean change: –2.5 mg/dL, range: –44 to +16 mg/dL, respectively, P<0.001), but no significant changes in TG and apo C-III concentrations (Figure 1).

Relative to baseline, no significant changes in plasma HDL-C, apo A-I, and HDL subpopulation levels occurred in the placebo arm. Relative to placebo, estrogen alone caused significant increases in mean plasma concentrations of HDL-C, apo A-I, and the HDL subpopulations pre β 1, α 1, α 2, α 3, and pre α 1 (Figure 2). Relative to placebo, the combination treatment was

associated with significant increases in plasma concentrations of HDL-C, apo A-I, and the HDL subpopulations $\alpha 1$ and $\alpha 2$ (Figure 2).

The changes in plasma RLP-C concentrations were significantly and positively correlated with changes in plasma TG and pre $\beta 1$ (Spearman's $\rho=0.339$ and 0.181 , respectively, $P<0.004$), and inversely associated with changes in plasma $\alpha 1$ and $\alpha 2$ concentrations ($\rho=-0.302$ and -0.200 , respectively, $P<0.002$).

The changes in plasma RLP-C concentrations were significantly associated with changes in minimal coronary artery diameter in the whole population ($P<0.008$), reflecting faster progression of coronary atherosclerosis in women who experienced greater reductions in RLP-C concentrations on trial (0.0038 mm average reduction in MMD per 1 mg/dL reduction in RLP-C levels) (Table 3). Since estrogen and the combination treatment caused similar changes in RLP-C and HDL subpopulations, these two groups were combined in further analyses. When only subjects in active treatment arms were included in the analysis, the association between RLP-C and coronary disease progression remained highly significant (Table 4). Moreover, women in the tertile of larger HT-related reductions in RLP-C concentrations had significantly faster coronary atherosclerosis progression ($P<0.016$, after adjustment) (Figure 3). No association between change in minimal lumen diameter and change in RLP-C concentrations was observed in the placebo group ($P=0.52$). Changes in plasma TG and apo C-III levels were not associated with progression in coronary artery disease either in the whole population (Table 3) or in subjects in the active treatment arms (Table 4).

Changes in plasma apo A-I and HDL-C concentrations did not predict coronary atherosclerosis progression. However, changes in pre $\beta 1$ HDL particle concentrations were associated with changes in minimum coronary artery diameter, suggesting faster coronary artery disease progression in women who had greater reductions in this HDL particle during the trial (Table 3). This association was still significant after exclusion of subjects on placebo (Table 4). While faster progression of coronary atherosclerosis was observed in women in the tertile of greater reductions in pre $\beta 1$, this association was no longer significant after adjustment for other CHD risk factors ($P=0.11$). None of the other HDL particles were predictors of CHD progression either in the whole population (Table 3) or in the active treatment groups (Table 4). In the placebo group, changes in pre $\beta 1$ particle concentrations were not related to changes in mean minimal diameter over the course of the trial ($P=0.92$). In the placebo group, a trend toward an association of the reduction in mean minimal lumen diameter with increases in $\alpha 3$ (estimate: -0.0034 , $P=0.08$), and reductions in pre $\alpha 2$ (estimate: 0.0244 , $P=0.06$), and pre $\alpha 3$ (estimate: 0.0179 , $P=0.09$) concentrations was observed. These latter associations are in agreement with the suggested proatherogenic role of $\alpha 3$ and antiatherogenic role of pre α particles (15,16).

DISCUSSION

Clinical intervention trials of lipid-lowering medications have clearly established the causative association between LDL-C lowering and CHD risk reduction (23). The importance of HDL-C raising in the prevention of CHD has also been documented (24–26). Based on the documented LDL-C lowering and HDL-C raising effects of most HT preparations and the CHD risk reduction with HT in observational studies, it had been postulated that HT could lower the risk of CHD in postmenopausal women in part through its effects on plasma lipoproteins. However, randomized clinical trials of HT have consistently shown lack of protection against CHD in postmenopausal women, both in secondary (1,3,4) and primary prevention (5,6) settings, in spite of documented beneficial changes in plasma lipid levels with HT. In agreement with the results of other randomized HT trials, the ERA study did not show cardiovascular benefit of HT in postmenopausal women with established CHD (2).

Effect of HT on plasma remnant lipoproteins

A significant reduction in plasma RLP-C concentrations was observed in both active treatment arms of the study, in agreement with most (27–30) but not all HT studies (31). In these studies, the reduction in plasma remnant lipoprotein concentrations occurred in the context of no change or elevation in plasma TG levels. Estrogen administration may cause elevations in plasma TG concentrations by increasing hepatic synthesis and secretion of TG-rich very low density lipoproteins (VLDL) (32). Estrogen also increases the hepatic expression of the LDL receptor (33). The reduction in remnant lipoproteins observed with HT may be attributed to their increased uptake via the interaction of apo E on their surface with the hepatic LDL receptor.

Effect of HT on plasma HDL subpopulations

Significant increases in HDL-C and apoA-I levels were observed in both active treatment arms of the ERA trial. HT increases HDL-C and apo A-I in part by increasing apo A-I production (34). The mechanism for the HT-related change in HDL subpopulation profile is not known, but it may be explained by both an increase in apo A-I synthesis and changes in the activity of enzymes and receptors involved in the continuous remodeling of HDL in plasma: estrogen lowers the expression of both the hepatic HDL receptor scavenger receptor class B type I (SR-BI) (35), which is likely to result in an increase in large HDL particles (36), and the enzyme hepatic lipase, which can further promote the formation of large HDL particles and also the increase in pre β 1 particle.

Relation of changes in lipoprotein subpopulations to progression in coronary disease

The change in minimal coronary artery diameter over the course of the study was similar in the three treatment arms (2).

Contrary to expectations, women who experienced greater reductions in atherogenic remnant lipoproteins on HT showed faster progression in coronary disease. Similarly, women who experienced greater reductions in HDL pre β 1 concentrations during HT had faster progression of coronary atherosclerosis.

Remnant lipoproteins are atherogenic, especially in women (14,37). In the same ERA population, we have previously documented a significant positive association between elevated levels of remnant lipoproteins and extent of coronary artery disease at baseline (38). To our knowledge, only the WAVE study had examined the association between changes in RLP-C concentrations and progression of CHD in postmenopausal women on HT (31). However, in the WAVE study there were no significant changes in RLP-C with HT and no association with CHD progression was detected.

It is well established that higher HDL-C levels are associated with a reduction in CHD risk (39). Moreover, several studies have indicated that higher plasma α 1 and α 2 and lower pre β 1 particle concentrations are better predictors of lower CHD risk than HDL-C (15,16,40). In contrast, we observed faster progression in disease in subjects who experienced greater HT-related reductions in pre β 1 concentrations. Pre β 1 is a small, lipid-poor apo A-I containing particle that acquire cholesterol through interaction with ABCA1, a transporter on plasma cell membranes, and it has been proposed that elevations in pre β 1 reflect an impairment in HDL maturation (15).

The mechanism by which HT causes lipoprotein changes is different from that of other lipid-lowering medications that have been proven to beneficially influence CHD risk. Both statins and niacin remodel HDL by increasing the large α 1 and α 2 particles and reducing the small α 3 and pre β 1 particles (41,42). In contrast, HT increased the concentration of both large and small HDL particles. It is possible that HT promotes the formation of dysfunctional HDL via

different mechanisms: by promoting the formation of TG-enriched HDL (43), which have reduced anti-atherogenic potential; by reducing the hepatic SR-BI expression and SR-BI-mediated hepatic selective cholesterol removal; and by increasing HDL pro-inflammatory properties in the setting of systemic inflammation (44). Another possibility is that the degree of change in lipoprotein levels during HT is an indirect marker of “estrogen sensitivity” in women with CHD, identifying subjects with an increased inflammatory response to HT. In the ERA study, women in the tertile with greater RLP-C reductions had significantly greater on-trial changes in inflammatory markers than women in the other two tertiles (mean change in C-reactive protein: 0.44 µg/ml vs 0.28 µg/ml, respectively, $P<0.03$; mean change in intercellular adhesion molecule-1: -20 ng/ml vs -30 ng/ml, respectively, $P<0.01$). The molecular mechanisms that lead to an increased expression of LDL-R, apo A-I gene, and inflammatory genes by estrogen mostly involve non-genomic effects (33,45). It is possible that estrogen causes the activation of gene transcription in lipid metabolism, coagulation, inflammation and plaque instability through mechanisms that are not mediated by the classical estrogen response element. Accordingly, women with “estrogen sensitivity” will have greater HT response in terms of plasma lipid levels, but also inflammation and thrombogenicity. In women with CHD, the harmful effects on coagulation, inflammation, and plaque instability can override the beneficial effects on lipid metabolism, while in women without CHD the beneficial changes in lipoproteins would prevail. In support of this, recent evidence indicates that HT promotes the progression of CHD in native coronary arteries but slows the disease in saphenous vein grafts in postmenopausal women with CHD, supporting the dual and opposite role of estrogen (46).

Even though our analyses were adjusted for covariates known to influence CHD risk, including baseline coronary lumen diameter and baseline plasma remnant lipoprotein cholesterol and preß1 levels, we cannot rule out the presence of an unidentified confounder. In addition, multiple testing may have increased the likelihood of false positives.

It is unclear to what extent our results might be extrapolated to other estrogen replacement regimens differing in administration route and composition.

Clinical implications and conclusions

The mechanism by which estrogen lowers LDL-C and increases HDL-C concentrations is different from that of other lipid-lowering medications and may be the key to the documented lack of protection observed with estrogen replacement in postmenopausal women with CHD. Our results do not support the use of HT in postmenopausal women for the prevention of CHD progression.

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Figure 1.

Changes in plasma TG (panel A), apo C-III (panel B), and RLP-C (panel C) concentrations in the placebo (white), estrogen (light gray), and estrogen plus progestin (dark gray) arms of the ERA study; * $P < 0.001$, relative to changes in the placebo arm. Data expressed as mean change $\pm$ SE.

Figure 2.

Changes in plasma HDL-C, apo A-I, and HDL subpopulations in the placebo (upper panel), estrogen (middle panel), and estrogen + progestin (lower panel) arms of the ERA trial. HDL subpopulations are expressed as mg/dL of plasma apo A-I. * $P < 0.001$, relative to changes in the placebo arm. Data expressed as mean change $\pm$ SE.

Figure 3.

Mean changes in mean minimum coronary artery diameter by tertiles of changes in plasma RLP-C levels ($P<0.016$) in women randomized to HT ($N=152$).

Table 1
Characteristics of ERA participants at baseline (N=256).

	Placebo	Estrogen	Estrogen+Progestin	P value
Age, years *	66 (± 7)	66 (± 8)	65 (± 7)	0.82
BMI, kg/m ²	30.2 (± 8.6)	29.1 (± 6.0)	29.7 (± 8.2)	0.66
Race, %				0.33
White	82	83	85	
Black	12	12	15	
Other	6	5	0	
Smoking, %	22	16	18	0.31
Diabetes, %	33	21	26	0.24
Lipid-lowering medications, %	37	34	38	0.70
Hypertension, %	70	64	69	0.61
Plasma lipids, mg/dL				
TC	215 (± 40)	214 (± 47)	220 (± 38)	0.45
TG	207 (± 122)	196 (± 115)	170 (± 82)	0.10
LDL-C	134 (± 36)	133 (± 40)	138 (± 34)	0.40
Minimal coronary artery Diameter, mm	1.95 (± 0.75)	1.94 (± 0.72)	1.89 (± 0.74)	0.31

Data expressed as mean ($\pm$ SD)

Table 2
Plasma concentrations of RLP-C, apo C-III, apo A-I, and HDL subpopulations at baseline by randomization group.

	Placebo	Estrogen	Estrogen+Progestin	P value
<i>mg/dL</i>				
RLP-C	14.2 (±14.5)	12.5 (±9.1)	11.7 (±9.3)	0.42
Apo C-III	14.1 (±4.5)	14.3 (±5.8)	13.7 (±5.0)	0.66
HDL apo C-III	9.9 (±4.8)	9.78 (±3.4)	9.5 (±4.3)	0.68
Apo A-I	125 (±21)	125 (±17)	128 (±20)	0.55
HDL-C	43 (±12)	43 (±11)	46 (±14)	0.14
HDL supopulations				
preβ1	23.5 (±9.8)	23.8 (±8.3)	22.3 (±8.3)	0.55
preβ2	2.1 (±1.4)	2.1 (±1.3)	2.3 (±1.1)	0.69
α1	12.5 (±8.8)	11.7 (±7.1)	13.8 (±8.8)	0.27
α2	37.1 (±10.9)	37.8 (±10.0)	38.8 (±10.1)	0.58
α3	38.3 (±9.5)	38.7 (±8.0)	38.0 (±8.7)	0.86
preα1	2.4 (±1.9)	2.3 (±2.2)	3.0 (±2.3)	0.05
preα2	4.1 (±1.9)	4.2 (±2.0)	4.7 (±2.2)	0.11
preα3	4.7 (±2.1)	4.8 (±1.8)	5.0 (±1.9)	0.29

values are mean (±SD)

HDL subpopulations expressed as mg/dL of total plasma apo A-I.

Table 3

Associations between changes in plasma lipoprotein levels and progression of coronary atherosclerosis (all arms).

Variable ¹ (change)	Change in minimum diameter	
	Estimate ²	P
RLP-C	0.0038	0.008
Apo C-III	0.0018	0.57
HDL apo C-III	0.0020	0.57
TG	0.0000	0.96
Apo A-I	−0.0002	0.74
HDL-C	−0.0025	0.10
HDL supopulations		
preβ1	0.0038	0.04
preβ2	−0.0011	0.46
α1	−0.0009	0.58
α2	−0.0014	0.31
α3	−0.0026	0.10
preα1	0.0035	0.64
preα2	0.0034	0.69
preα3	0.0092	0.21

¹ Change in variables expressed in mg/dL² Beta-hat from mixed-model analysis adjusted for mean minimal diameter at baseline, location in the artery segment, age, BMI, race, smoking, hypertension, diabetes, use of lipid-lowering medications, and prior percutaneous angioplasty

Table 4

Associations between changes (and percent changes) in plasma lipoproteins and coronary atherosclerosis progression in subjects in the active treatment arms only.

Variable ¹ (change)	Change in minimum diameter		Variable(% change)	Change in minimum diameter	
	Estimate ²	P		Estimate ²	P
RLP-C	0.0074	0.0001	RLP-C	0.0011	0.007
Apo C-III	0.0030	0.47	Apo C-III	0.0026	0.54
HDL apo C-III	0.0046	0.29	HDL apo C-III	0.0002	0.57
TG	0.0000	0.91	TG	0.0000	0.99
Apo A-I	-0.0006	0.47	Apo A-I	-0.0004	0.65
HDL-C	-0.0045	0.02	HDL-C	-0.0016	0.05
HDL subpopulations			HDL subpopulations		
preβ1	0.0060	0.01	preβ1	0.0010	0.07
preβ2	-0.0138	0.47	preβ2	0.0000	0.75
α1	-0.0026	0.21	α1	-0.0001	0.37
α2	-0.0031	0.08	α2	-0.0001	0.08
α3	-0.0013	0.55	α3	-0.0003	0.66
preα1	0.0008	0.93	preα1	0.0000	0.27
preα2	-0.0046	0.68	preα2	-0.0002	0.56
preα3	0.0129	0.19	preα3	0.0002	0.65

¹ Change in variables expressed in mg/dL

² Beta-hat from mixed-model analysis adjusted for mean minimal diameter at baseline, location in the artery segment, age, BMI, race, smoking, hypertension, diabetes, use of lipid-lowering medications, and prior percutaneous angioplasty.