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Effect of hormone replacement therapy on plasma lipoprotein levels and coronary atherosclerosis progression in postmenopausal women according to type 2 diabetes mellitus status

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Abstract

Type 2 diabetes mellitus is associated with dyslipidemia and with an increased risk of coronary heart disease (CHD). Our objective was to compare the effects of hormone replacement therapy (HRT) on plasma lipoproteins and coronary disease progression in postmenopausal women with and without diabetes. Study subjects were participants in the Estrogen Replacement and Atherosclerosis trial, a placebo-controlled, randomized trial of HRT (conjugated equine estrogen 0.625 mg/d with or without medroxyprogesterone acetate 2.5 mg/d) in postmenopausal women with established CHD (mean age, 65 ± 7 years). Plasma remnant lipoprotein levels and high-density lipoprotein (HDL) subpopulation levels were measured at baseline and year 1. Quantitative coronary angiography was assessed at baseline and at follow-up. At baseline, remnant lipoprotein levels were significantly higher and HDL cholesterol (HDL-C) levels were significantly lower in diabetic women than in women without diabetes. Hormone replacement therapy lowered remnant lipoproteins and increased HDL-C and large HDL particle levels in both groups. However, during HRT, levels of these parameters were still significantly worse in diabetic women than in nondiabetic women. A significant interaction between HRT and diabetes status, with greater increases in plasma atheroprotective HDL α1 particles in nondiabetic women than in diabetic women during HRT, was observed. Coronary heart disease progressed significantly more in women with diabetes than in women without diabetes. Our findings indicate that diabetes attenuates the HRT-related increase in atheroprotective HDL α1 particles. Faster progression of coronary atherosclerosis in women with diabetes could be mediated in part by a worse lipoprotein profile in these women than in women without diabetes, both before and during HRT.

1. Introduction

Coronary heart disease (CHD) is the main cause of death in postmenopausal women [1]. Type 2 diabetes mellitus increases the risk of CHD [2,3], and there is some evidence that the risk conferred by diabetes is greater in women than in men [4]. The increased risk of CHD with diabetes is in part mediated by the associated dyslipidemia, characterized by elevated plasma triglyceride (TG) and reduced high-density lipoprotein cholesterol (HDL-C) levels, in addition to elevated small dense low-density lipoprotein (LDL) levels [5]. Other important mediators of increased CHD risk in diabetes are obesity, hypertension, and increased inflammation. The typical dyslipidemia observed in diabetes is due to the insulin-resistance state, which causes increased hepatic synthesis of TG-rich lipoproteins and a faster clearance of HDL [6-8].

In randomized clinical trials conducted mostly in older women, hormone replacement therapy (HRT) has been shown either to offer no protection against CHD [9,10] or to increase the risk of CHD [11,12]. Use of HRT in younger postmenopausal women may reduce the risk of CHD [13,14]. Although HRT is no longer prescribed for the prevention of CHD, postmenopausal women are still

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prescribed HRT for the relief of menopausal symptoms. By lowering plasma LDL cholesterol (LDL-C) and increasing HDL-C levels, HRT has the potential of improving the dyslipidemia associated with diabetes, with the exception of potentially further increasing plasma TG levels. In a randomized study of HRT in postmenopausal women with CHD, it was shown that coronary artery disease progression was faster in women with glucose intolerance randomized to HRT than in normoglycemic women randomized to HRT [15].

The aims of this study were to compare the lipoprotein subpopulation response to HRT in nondiabetic and diabetic women with established CHD.

2. Methods

2.1. Study design

Subjects were postmenopausal women participating in the Estrogen Replacement and Atherosclerosis (ERA) trial [9,16]. Women with established *CHD*, defined as at least 30% stenosis of at least one epicardial coronary artery by quantitative coronary angiography, were enrolled into the study. Exclusion criteria were as follows: history of uncontrolled diabetes or hypertension, deep vein thrombosis or pulmonary embolism, kidney disease, symptomatic gallstones, or TG levels greater than 400 mg/dL. The study had a randomized, placebo-controlled, double-blind design and consisted of 3 parallel phases: (*a*) oral conjugated equine estrogen (CEE 0.625 mg/d, as Premarin, Wyeth-Ayerst, Philadelphia, PA), (*b*) oral CEE and medroxyprogesterone acetate (CEE 0.625 mg/d and MPA 2.5 mg/d, as Prempro, Wyeth-Ayerst), and (*c*) placebo. Participants were followed for a mean of 3.2 years. Of the 309 enrolled subjects, paired plasma samples at baseline and at year 1 for the assessment of plasma lipoprotein subpopulations were available in 250 women. Paired baseline and follow-up coronary angiography assessments were available in 217 women. *Diabetes* was defined as use of hypoglycemic medications or a fasting glucose level of at least 126 mg/dL.

2.2. Measurement of plasma lipids, remnant lipoproteins, and HDL subpopulations

Fasting blood samples were obtained from study participants at the baseline and the year 1 visits. Blood was drawn in tubes containing 0.1% EDTA, and plasma was separated by centrifugation at 1000*g* for 30 minutes at 4 °C. Plasma total cholesterol (TC) and TG concentrations were measured by automated assays [17]. High-density lipoprotein cholesterol levels were measured after precipitation of apolipoprotein (apo) B–containing lipoproteins with heparin-manganese [18]. Low-density lipoprotein cholesterol levels were calculated with the Friedewald formula [19]. Plasma apo A-I and apo C-III levels 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]. High-density lipoprotein was separated into 8 subpopulations (pre-β1, pre-β2, α1, α2, α3, pre-α1, pre-α2, and pre-α3) according to charge, size, and composition. The concentration of each HDL subpopulation was calculated by multiplying its percentage by the total plasma apo A-I concentration and was expressed as milligrams per deciliter of apo A-I.

Plasma C-reactive protein (CRP) levels were measured with an enzyme-linked immunosorbent assay (American Laboratory Products, Windham, NH) [23].

2.3. Coronary angiography

Quantitative coronary angiography was performed at baseline and after approximately 3.2 years of follow-up, as previously described [9,16]. Analysis of angiograms was performed in pairs with a previously validated system of cine-projection (SME 3500; Sony, Park Ridge, NJ). The mean intraoperator 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 [9,16].

2.4. Statistical analysis

All analyses were based on intent-to-treat. Skewed variables were log-transformed before analysis. Changes in plasma lipid and lipoprotein variables were calculated as $\text{value}_{\text{year 1}} - \text{value}_{\text{baseline}}$. The change in mean minimum coronary artery diameter (MMD) was calculated as $\text{MMD}_{\text{follow-up}} - \text{MMD}_{\text{baseline}}$. We have previously reported a similar effect of CEE and CEE + MPA on plasma lipoproteins [24]. Therefore, women randomized to either one of these two treatments were combined in one group and compared with placebo-treated women. The effects of treatment on plasma concentrations of lipids, apolipoproteins, and lipoprotein subspecies were tested by multiple regression analysis. The model was adjusted for age, race, smoking, and use of lipid-lowering medications. Diabetes and the interaction between diabetes and treatment were also included in the model. Analysis of covariance was used to test the hypothesis of an association between changes in MMD and changes in lipoprotein subspecies. The model was adjusted for baseline minimal coronary lumen diameter, age, body mass index (BMI), race, smoking, use of lipid-lowering medications, A P value $< .05$ was set as statistically significant. The magnitude of treatment effect, or effect size, was assessed with the formula by Cohen: $d = M_1 - M_2/\text{SD}_{\text{pooled}}$, where M_1 is the mean change during placebo, M_2 is the mean change during HRT, and $\text{SD}_{\text{pooled}}$ is $= \sqrt{[(\text{SD}_1^2 + \text{SD}_2^2)/2]}$ [25].

Table 1
Baseline characteristics of CHD women without and with diabetes in the ERA trial

	No diabetes (n = 182)	Diabetes (n = 68)	P*
Age, y	66 (7)	64 (7)	.01
BMI, kg/m^2	28.22 (7.7)	33.8 (6.7)	.0001
Race, %			.001
White	88	69	
Black	8	26	
Other	4	5	
Smoking, %	24	16	.24
Lipid-lowering medications, %	33	34	.88
Plasma glucose, mg/dL	99 (16)	170 (51)	.0001
Systolic blood pressure, mm Hg	134 (18)	138 (16)	.13
Diastolic blood pressure, mm Hg	74 (8) (n = 158)	75 (9) (n = 59)	.52
Min lumen diameter, mm	1.93 (0.33)	1.90 (0.40)	.49

Data are mean (SD).
* *P* value; Mann-Whitney test for continuous variables and Fisher exact test for categorical variables.

According to Cohen [25], an absolute *d* value of less than 0.3 is defined as a *small effect size*, whereas a *d* greater than 0.8 is considered a *large effect size*.

3. Results

At baseline, 182 of the ERA subjects participating in this study were free of diabetes; and 68 had type 2 diabetes mellitus (Table 1). Women with diabetes were significantly younger and had a higher BMI than women without diabetes. The percentage of African Americans was higher in the diabetes group than in the group without diabetes. Baseline fasting plasma glucose levels were higher in diabetic than nondiabetic women, but blood pressure and coronary artery mean minimum lumen diameter were similar between the 2 groups of women.

Plasma TG, RLP-C, and apo C-III levels, but not plasma TC or LDL-C levels, were significantly higher in diabetic than in nondiabetic women at baseline (Table 2). Plasma HDL-C levels were significantly lower in diabetic women. However, no differences were observed in plasma apo A-I levels or in the apo A-I–containing HDL subpopulation profile between the 2 groups of women, with the exception of a slight elevation in levels of small pre-α3 particles in diabetic women (Table 2). Plasma CRP levels were significantly higher in women with diabetes than in nondiabetic women (Table 2). At year 1, after adjustment for treatment effects, the differences in plasma TG, remnant lipoproteins, and HDL-C between subjects without and with diabetes were even more marked. In addition, at year 1, the mean plasma concentration of HDL α2 particles was significantly lower in diabetic than in nondiabetic women (Table 2). However, the difference in plasma CRP levels was no longer present.

In a multiple regression analysis adjusted for age, race, use of lipid-lowering medications, and smoking, the on-trial changes in TC and LDL-C levels were significantly associated with treatment (Table 3). Hormone replacement therapy did not significantly affect plasma TG or apo C-III levels. Nevertheless, plasma RLP-C levels were significantly reduced by HRT. Plasma HDL-C and apo A-I levels and the

Table 2
Baseline and year-1 plasma lipid, lipoprotein, and CRP levels in CHD women without and with diabetes in the ERA trial

	Baseline			Year 1		
	No diabetes (n = 182)	Diabetes (n = 68)	P*	No diabetes (n = 182)	Diabetes (n = 68)	P†
	mg/dL	mg/dL		mg/dL	mg/dL	
TC	217 (40)	218 (46)	.50	209 (30)	211 (42)	.63
LDL-C	137 (35)	133 (43)	.09	122 (34)	123 (39)	.45
TG	184 (104)	225 (120)	.004	188 (116)	238 (140)	.0001
RLP-C	11.8 (9.9)	15.5 (12.3)	.007	10.8 (11)	13.7 (10.7)	.003
Apo C-III	13.7 (4.7)	15.8 (6.1)	.02	13.6 (4.6)	15.9 (5.9)	.004
HDL-C	45 (12)	42 (13)	.04	51 (15)	45 (14)	.004
Apo A-I	126 (19)	126 (19)	.80	140 (24)	135 (24)	.14
HDL subpopulations						
Pre-β1	23.5 (9.1)	22.8 (8.1)	.59	24.1 (8.7)	23.6 (9.2)	.56
Pre-β2	2.0 (1.2)	2.3 (1.4)	.46	2.1 (1.3)	2.6 (1.7)	.18
α1	12.9 (8.1)	12.2 (8.8)	.42	17.4 (10.4)	15.4 (10.7)	.11
α2	38.3 (10.6)	37.0 (9.7)	.25	43.2 (12.2)	38.9 (10.2)	.02
α3	38.0 (8.8)	39.3 (8.8)	.19	39.8 (9.9)	40.6 (7.3)	.15
Pre-α1	2.6 (2.2)	2.5 (2.0)	.96	3.4 (2.6)	3.1 (2.4)	.98
Pre-α2	4.3 (1.9)	4.5 (2.2)	.82	5.1 (2.3)	4.8 (2.2)	.35
Pre-α3	4.7 (1.8)	5.3 (2.1)	.02	4.8 (2.0)	5.3 (2.3)	.09
CRP	0.52 (0.68)	0.79 (0.74)	.03	0.77 (1.20)	0.76 (0.59)	.09

Mean (SD).
* *P* value, model covariates: age, race, smoking, and use of lipid-lowering medications.
† Model includes all covariates in * and treatment. Skewed variables were log-transformed before analysis.

Table 3
Changes in plasma lipid, lipoprotein, and CRP levels, and coronary atherosclerosis progression in the placebo and the HRT arms of the ERA study by diabetes status

	No diabetes			Diabetes			P* (treatment)	$P^{\dagger}$ (diabetes)	$P^{\ddagger}$ (interaction)
	Placebo (n = 59)	HRT (n = 123)	Effect size d	Placebo (n = 27)	HRT (n = 41)	Effect size d			
TC, mg/dL	3.2 (37)	−11.6 (40)	0.38	−1.9 (30)	−7.5 (37)	0.16	.02	.69	.45
LDL-C, mg/dL	−0.2 (31)	−18.5 (39)	0.52	7 (21)	−14.5 (42)	0.64	.0001	.13	.86
TG, mg/dL	6.7 (89)	1.4 (69)	0.07	7.0 (128)	20.5 (124)	−0.11	.86	.12	.43
RLP-C, mg/dL	1.7 (9)	−2.3 (6.2)	0.53	−0.6 (8)	−2.6 (10.1)	0.22	.001	.64	.42
Apo C-III, mg/dL	0.3 (3.4)	−0.2 (3.3)	0.15	−0.2 (3.3)	0.6 (4.3)	−0.21	.96	.76	.15
HDL-C, mg/dL	1.8 (7)	7.7 (8.9)	−0.74	0.4 (6)	4.6 (7.2)	−0.63	.0001	.02	.38
Apo A-I, mg/dL	1.8 (17)	19.5 (20)	−0.95	1.2 (12)	13.9 (18)	−0.83	.0001	.14	.21
HDL subpopulations, mg/dL									
Pre-β1	−1.1 (6.8)	1.5 (6.4)	−0.39	−2.0 (7.2)	2.7 (7.5)	−0.64	.0001	.63	.39
Pre-β2	−0.1 (0.7)	0.2 (0.8)	−0.40	0.2 (1.0)	0.3 (1.1)	−0.10	.06	.33	.38
α1	0.5 (4.5)	6.5 (7.2)	−0.99	2.3 (5.4)	3.9 (8.3)	−0.23	.0001	.38	.02
α2	0.4 (8.4)	7.0 (9.1)	−1.07	0.8 (5.9)	2.7 (8.7)	−0.26	.0001	.07	.06
α3	0.4 (7.9)	2.4 (7.5)	−0.26	−0.9 (7.2)	2.9 (7.8)	−0.50	.02	.70	.63
Pre-α1	0.2 (1.4)	1.1 (1.6)	−0.60	0.5 (1.5)	0.8 (1.8)	−0.18	.0001	.65	.09
Pre-α2	0.6 (1.5)	0.9 (1.4)	−0.21	0.2 (1.7)	0.4 (1.7)	−0.12	.14	.03	.86
Pre-α3	0.5 (1.9)	−0.1 (1.5)	0.35	0.1 (2.0)	0.0 (1.9)	0.05	.04	.53	.37
CRP, mg/dL	−0.05 (1.43) (n = 49)	0.38 (1.10) (n = 108)	−0.34	−0.18 (0.54) (n = 22)	0.06 (0.58) (n = 37)	−0.48	.01	.08	.52
Mean min lumen diameter, mm	−0.09 (0.19)	−0.08 (0.18)	−0.05	−0.19 (0.18)	−0.14 (0.26)	−0.22	.44	.04	.46

Mean(SD). Effect size: Cohen d.
* P value for treatment effect, model adjusted for age, race, smoking, and use of lipid-lowering medications.
† P value for diabetes effect, model as above.
‡ P value for the interaction between treatment and diabetes, model as above.

HDL subpopulations pre-β1, α1, α2, α3, and pre-α1 were significantly increased by HRT. Overall, HRT caused similar and significant changes in plasma lipoproteins in both diabetic and nondiabetic women; but the changes in HDL-C and pre-α2 particle levels were significantly lower in women with diabetes than those in healthy women. There was a statistically significant interaction ($P < .02$) between HRT and diabetes status for the change in HDL α1 particle levels, with lower increases in α1 levels in diabetic women on HRT, relative to placebo, than in women without diabetes (Table 3). A trend for a similar interaction between HRT and diabetes was also observed for α2 particles. This is also indicated by the large effect size for α1 and α2 in nondiabetic but small effect size in diabetic women. The observed interaction was not affected by the type of HRT, as similar changes in HDL, apo A-I, α1, and α2 particle levels were observed in the CEE and CEE + MPA arms of the study when analyzed by diabetes status (Fig. 1).

Plasma CRP levels were significantly increased by HRT, relative to placebo; but there was no interaction between treatment and diabetes status (Table 3).

Coronary artery atherosclerosis progressed significantly more in diabetic women than in healthy women, but HRT treatment did not affect the degree of progression in either group.

We have previously reported in this population a significant association of on-trial changes in remnant lipoprotein cholesterol and HDL pre-β1 levels with progression of coronary atherosclerosis [24]. As shown in Table 4,

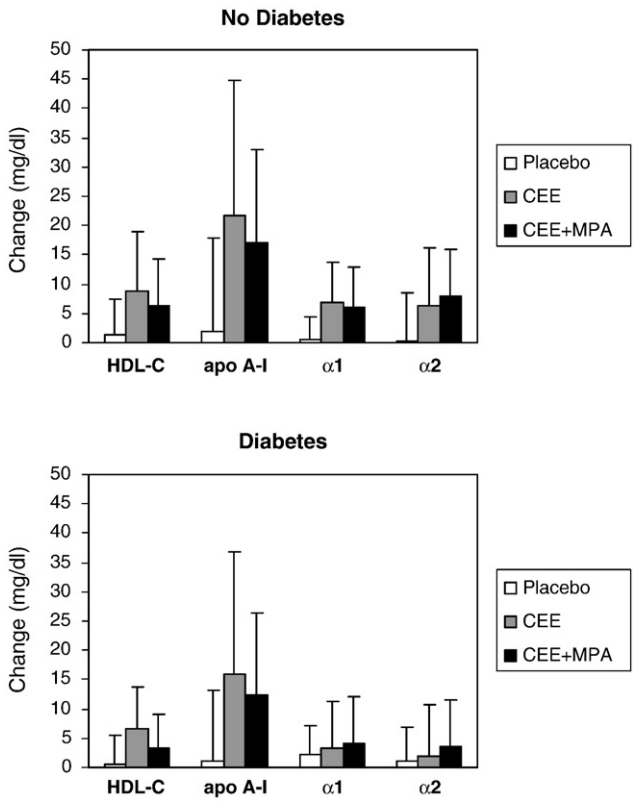

Fig. 1. Changes in HDL-C, apo A-I, and α1 and α2 particles according to treatment phase in postmenopausal women without diabetes (upper panel) and with diabetes (lower panel). Data shown as mean changes and SD.

Table 4
Association between progression in coronary atherosclerosis and change in plasma lipoproteins in women in the HRT arms of the ERA study

	All		No diabetes		Diabetes	
	B	*P*	*B*	*P*	*B*	*P*
RLP-C	0.005	.008	0.005	.05	0.005	.11
Apo A-I	0.001	.20	0.000	.68	0.003	.17
HDL-C	−0.001	.77	−0.001	.53	0.000	.96
HDL subpopulations						
Pre-β1	0.005	.02	0.005	.06	0.007	.11
α1	0.002	.40	0.001	.81	0.004	.37
α2	0.000	.85	−0.002	.34	0.006	.18

B: unstandardized coefficient; this provides a measure of the change in minimum lumen diameter (in millimeters) per each 1-mg/dL change in dependent variable.

although these associations were no longer significant when analyses were carried out separately in subjects without or with diabetes, the magnitude of the effect was similar in the 2 groups.

4. Discussion

Type 2 diabetes mellitus significantly increases the risk of CHD [2]. In this study, women with diabetes had a higher BMI and were more likely to be African Americans. These findings are in agreement with the current literature [2,26]. Mean minimum coronary artery diameter was similar in diabetic and nondiabetic women; but diabetic women were significantly younger, indicating earlier disease with diabetes.

Subjects with diabetes characteristically display higher plasma TG and lower HDL-C levels than healthy subjects. The increase in plasma TG levels is caused in part by an increased synthesis of TG-rich very low-density lipoproteins (VLDLs) by the liver [7]. In diabetes, insulin resistance promotes the release of free fatty acids from adipose tissue, resulting in greater hepatic availability of substrate for VLDL synthesis [27]. In addition, a reduction in TG-rich lipoprotein clearance has been observed in insulin-resistance states, which is due to an increase in plasma levels of apo C-III, an inhibitor of lipoprotein lipase [28]. The combination of increased synthesis and delayed clearance results in higher concentrations of circulating TG-rich and remnant lipoproteins and an increased atherogenic potential. Triglyceride-rich lipoproteins also act as substrate for the cholesteryl ester transfer protein: in hypertriglyceridemic states, the increased cholesteryl ester transfer protein activity, coupled with an increase in the expression of hepatic lipase, leads to increased catabolism of the large HDL particles, resulting in decreased overall HDL size and HDL-C levels [8]. Likewise, in our study, women with diabetes had significantly higher plasma levels of TG and remnant lipoproteins and lower plasma levels of HDL-C than nondiabetic women. These observations are consistent with findings from the Framingham Offspring study [29]. In addition, we found significantly higher plasma levels of apo C-III in women with diabetes, in agreement with previous findings [30,31]. Apolipoprotein C-III gene expression is down-regulated by insulin [32].

Women with diabetes had a faster progression of coronary disease relative to women without diabetes, but HRT did not affect disease progression in either group. Similarly to our findings, Howard et al [15] had found faster atherosclerosis progression in diabetic women than in nondiabetic women participating in the Women's Angiographic Vitamin and Estrogen Study. However, when the effect of HRT on coronary disease progression was examined in the latter study, it was found that HRT was associated with significantly faster atherosclerosis progression in coronary segments initially free of disease in diabetic women, but not in nondiabetic women [15]. These effects were accompanied by a worsening of CRP levels with HRT in women with diabetes but not in women without diabetes [15]. No effect of HRT was observed on the progression in diseased segments [15]. These results are somewhat in disagreement with our data, which indicate no interaction between HRT and diabetes status on CRP or atherosclerosis progression. However, in our analysis, we did not distinguish between diseased and nondiseased coronary artery segments. In a cross-sectional study of 212 diabetic and 411 nondiabetic postmenopausal women, Dubuisson et al [33] found greater carotid artery intima-media thickness in women with diabetes relative to women without diabetes. However, current and former HRT use was associated with lower intima-media thickness in the internal but not the common carotid artery, relative to nonusers, both in diabetic and nondiabetic women [33]. The observational nature of the study may explain the protective effect of HRT on atherosclerosis, as most observational studies have suggested a reduction in CHD risk with HRT [34].

Hormone replacement therapy has been shown to increase plasma TG levels by increasing the production of TG-rich VLDL particles [35]. Thus, HRT would be expected to worsen hypertriglyceridemia in diabetes. We found that women with diabetes had a nonsignificant increase in TG levels during HRT, but this was not statistically different from the effect of HRT on TG levels in women without diabetes. On the other hand, both groups of women experienced a significant, and similar in magnitude, reduction in remnant lipoproteins. A significant reduction in the postprandial elevation in TG levels in diabetic women treated with HRT, relative to placebo, has been reported [36]; and a reduction of intestinal TG-rich lipoproteins during treatment with estrogen has been reported in nondiabetic women [37]. The reduction in postprandial TG and in remnant lipoprotein levels with HRT could be mediated by a faster clearance of TG particles, either via enhanced receptor-mediated clearance or by increased lipolysis. Estrogen has been shown to increase the expression of hepatic LDL receptors in rats [38] and the expression of lipoprotein lipase in heart muscle in mice [39]. In our study,

apo C-III was not significantly modified by HRT, making it unlikely that apo C-III contributed to the reduction in remnant lipoproteins with HRT. Hormone replacement therapy has been shown to improve insulin resistance in postmenopausal women [13], but it is not known if this plays a role in the lipoprotein changes. Hormone replacement therapy is also known to reduce plasma LDL-C and increase plasma HDL-C levels. We found that HRT was effective in lowering LDL-C in women both with and without diabetes. In addition, HRT resulted in significant increases in plasma HDL-C and apo A-I levels.

During HRT, a significant increase in the level of the major HDL subpopulations was observed. We observed an interaction between treatment and presence of diabetes for the change in HDL particles, with a significantly smaller increase in large $\alpha 1$ particle levels and a trend for smaller increases in $\alpha 2$ particle levels by HRT in diabetic compared with nondiabetic women. Moreover, $\alpha 2$ particle levels were significantly lower and $\alpha 1$ particle levels tended to be lower in women with diabetes than in women without diabetes at year 1, after adjustment for treatment effects, suggesting that diabetes is associated both with lower levels of the large HDL particles and with a reduced response to HRT. The reduced increase in $\alpha 1$ and $\alpha 2$ particles following HRT treatment in diabetic women, compared with nondiabetic women, may be due to the fact that diabetic women had significantly higher TG levels than nondiabetic women and therefore an impaired HDL metabolism. It is well documented that high plasma levels of $\alpha 1$ and $\alpha 2$ particles are significantly associated with less atherosclerosis [40-42]. Therefore, it may be hypothesized that, in diabetic women, lower levels of large HDL particles lead to a less efficient reverse cholesterol transport and contribute to a faster atherosclerosis progression. The faster coronary atherosclerosis progression in diabetic women than in nondiabetic women is consistent with the increased risk of CHD conferred by the presence of diabetes and indicates that "beneficial" changes in plasma lipoproteins with HRT do not significantly retard the progression of atherosclerosis in diabetic women. Moreover, because posttreatment levels of TG-rich lipoprotein and HDL were still worse in diabetic than in nondiabetic women, these differences may still have contributed to the faster progression of CHD in diabetic women.

A limitation of this study is that our data do not provide definitive information but rather suggest provocative patterns that need to be more fully explored in larger data sets. Because HRT is still used by postmenopausal women for the relief of vasomotor symptoms, if diabetes does indeed attenuate the favorable effects of HRT on HDL particles, our results would shed additional light on the results of HRT clinical trials and provide additional motivation for glucose regulation in women contemplating HRT.

In conclusion, HRT-related changes in markers of CHD risk are significantly affected by the presence of abnormal glucose metabolism. More research is warranted to determine if normalization of glucose metabolism will lead to more favorable effects of HRT on lipoprotein profiles.

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References

[1] American Heart Association. Heart disease and stroke statistics—2008 update. Circulation 2008;117:e25-e146.
[2] The Expert Panel. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
[3] Manson JE, Colditz GA, Stampfer MJ, Willett WC, Krolewski AS, Rosner B, et al. A prospective study of maturity-onset diabetes and risk of coronary heart disease and stroke in women. Arch Int Med 1991; 151:1141-7.
[4] Huxley R, Barzi F, Woodward M. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective studies. BMJ 2006;332:73-8.
[5] Siegel RD, Cupples A, Schaefer EJ, Wilson WPF. Lipoproteins, apolipoproteins, and low-density lipoprotein size among diabetics in the Framingham offspring study. Metabolism 1996;45:1267-72.
[6] Olefsky JM, Farguhar JW, Reaven GM. Reappraisal of the role of insulin in hypertriglyceridemia. Am J Med 1974;57:551-60.
[7] Cummings MH, Watts GF, Umpleby AM, Hennessy TR, Naoumova R, Slavin BM, et al. Increased hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in NIDDM. Diabetologia 1995;38: 959-67.
[8] Frenais R, Ouguerram K, Maugeais C, Mahot P, Maugere P, Krempf M, et al. High density lipoprotein apolipoprotein AI kinetics in NIDDM: a stable isotope study. Diabetologia 1997;40:578-83.
[9] Herrington DM, Reboussin DM, Brosnihan B, Sharp PC, Shumaker SA, Snyder TE, et al. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. N Engl J Med 2000; 343:522-9.
[10] The Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy. The Women's Health Initiative randomized controlled trial. JAMA 2004;291:1701-12.
[11] Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321-33.
[12] Hulley SB, Grady D, Bush T, Furberg CD, Herrington DM, Riggs B, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 1998;280:605-13.
[13] Salpeter SR, Walsh JME, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. Diab Obes Metab 2006;8:538-54.

[14] Rossouw J, Prentice R, Manson JA, Wu L, Barad D, Barnabei V, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA 2007;297:1465-77.
[15] Howard BV, Hsia J, Ouyang P, Van Voorhees L, Lindsay J, Silverman A, et al. Postmenopausal hormone therapy is associated with atherosclerosis progression in women with abnormal glucose tolerance. Circulation 2004;110:201-6.
[16] Herrington DM, Reboussin DM, Klein K, Sharp PC, Shumaker SA, Snyder TE, et al. Estrogen Replacement and Atherosclerosis (ERA) study: study design and baseline characteristics of the cohort. Control Clin Trials 2000;21:257-85.
[17] McNamara JR, Schaefer EJ. Automated enzymatic standardized lipid analyses for plasma lipoprotein fractions. Clin Chim Acta 1987;166: 1-8.
[18] Burstein M, Samaille J. Sur un dosage rapide du cholesterol lie aux alpha- et aux beta-lipoproteines du serum. Clin Chim Acta 1960;5:609.
[19] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
[20] Contois J, McNamara J, Lammi-Keefe C, Wilson P, Massov T, Schaefer E. Reference intervals for plasma apolipoprotein A-I determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. Clin Chem 1996;42:507-14.
[21] McNamara JR, Shah PK, Nakajima K, Cupples LA, Wilson WPF, Ordovas JM, et al. Remnant lipoprotein cholesterol and triglyceride reference ranges from the Framingham Heart Study. Clin Chem 1998; 44:1224-32.
[22] Asztalos BF, Sloop CH, Wong L, Roheim PS. Two-dimensional electrophoresis of plasma lipoproteins: recognition of new apo A-I–containing subpopulations. Biochim Biophys Acta 1993;1169: 291-300.
[23] Lakoski SG, Brosnihan B, Herrington DM. Hormone therapy, C-reactive protein, and progression of atherosclerosis: data from the Estrogen Replacement on progression of coronary artery Atherosclerosis (ERA) trial. Am Heart J 2005;150:907-11.
[24] Lamon-Fava S, Herrington DM, Reboussin BA, Sherman M, Horvath KV, Schaefer EJ, et al. Changes in remnant and high-density lipoproteins associated with hormone therapy and progression of coronary artery disease in postmenopausal women. Atherosclerosis 2009;205:325-30.
[25] Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale (NJ): Lawrence Earlbaum Associates; 1988.
[26] Bowman SA. Socioeconomic characteristics, dietary and lifestyle patterns, and health and weight status of older adults in NHANES, 1999-2002: a comparison of Caucasians and African Americans. J Nutr Elderly 2009;28:30-46.
[27] Lewis G. Fatty acid regulation of very low density lipoprotein production. Curr Opin Lipidol 1997;8:146-53.
[28] Chan DC, Watts GF, Nguyen MN, Barrett P. Apolipoproteins C-III and A-V as predictors of very-low-density lipoprotein triglyceride and apolipoprotein B-100 kinetics. Arterioscler Thromb Vasc Biol 2006; 26:590-6.
[29] Schaefer EJ, McNamara JR, Shah PK, Nakajima K, Cupples LA, Ordovas JM, et al. Elevated remnant-like particle cholesterol and triglyceride levels in diabetic men and women in the Framingham Offspring Study. Diabetes Care 2002;25:989-94.
[30] Olivieri O, Bassi A, Stranieri C, Trabetti E, Martinelli N, Pizzolo F, et al. Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease. J Lipid Res 2003;44:2374-81.
[31] Cohn J, Patterson BW, Uffelman KD, Davignon J, Steiner G. Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. J Clin Endocrinol Metab 2004;89:3949-55.
[32] Altomonte J, Cong L, Harbaran S, Richter A, Xu J, Meseck M, et al. Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. J Clin Invest 2004;114:1493-503.
[33] Dubuisson JT, Wagenknecht LE, D'Agostino RB, Haffner S, Rewers M, Saad MF, et al. Association of hormone replacement therapy and carotid wall thickness in women with and without diabetes. Diabetes Care 1998;21:1790-6.
[34] Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of epidemiologic evidence. Prev Med 1991;20:47-63.
[35] Walsh BW, Schiff I, Rosner B, Greenberg LJ, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 1991;325: 1196-204.
[36] Friday KE, Dong C, Fontenot RU. Conjugated equine estrogen improves glycemic control and blood lipoproteins in postmenopausal women with type 2 diabetes. J Clin Endocrinol Metab 2001;86:48-52.
[37] Westerveld H, Kock LAW, Rijn HJM, Erkelens D, Bruin TWA. 17 beta-estradiol improves postprandial lipid metabolism in postmenopausal women. J Clin Endocrinol Metab 1995;80:249-53.
[38] Windler EET, Kovanen PT, Chao YS, Brown MS, Havel RJ, Goldstein JL. The estradiol-stimulated lipoprotein receptor in the rat liver. J Biol Chem 1980;255:10464-71.
[39] Liu D, Deschamps A, Korach KS, Murphy E. Estrogen-enhanced gene expression of lipoprotein lipase in heart is antagonized by progesterone. Endocrinology 2008;149:711-6.
[40] Asztalos BF, Cupples LA, Demissie S, Horvath KV, Cox CE, Batista M, et al. High-density lipoprotein subpopulation profile and coronary heart disease prevalence in male participants of the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 2004;24:2181-7.
[41] Asztalos BF, Collins D, Cupples LA, Demissie S, Horvath KV, Bloomfield HE, et al. Value of high-density lipoprotein (HDL) subpopulations in predicting recurrent cardiovascular events in the Veterans Affairs HDL Intervention Trial. Arterioscler Thromb Vasc Biol 2005;25:2185-91.
[42] Asztalos BF, Batista M, Horvath KV, Cox CE, Dallal G, Morse JS, et al. Change in alpha1 HDL concentration predicts progression in coronary artery stenosis. Arterioscler Thromb Vasc Biol 2003;23: 847-52.