Hormone-Replacement Therapy Increases Serum Paraoxonase Arylesterase Activity in Diabetic Postmenopausal Women

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The paraoxonase (PON1) enzyme is associated with high-density lipoproteins (HDL) in the blood and is low in patients with type 2 diabetes. Hormone-replacement therapy (HRT) can increase HDL cholesterol levels, but its effect on serum PON1 arylesterase activity is uncertain. The aim of the present study was to determine the effect of 6 months' HRT with conjugated equine estrogen and medroxyprogesterone acetate on serum PON1 arylesterase activity in postmenopausal women with type 2 diabetes. Serum PON1 activity was measured immediately before and at the end of the second arm of a randomized, placebo-controlled, crossover with washout study originally designed to test the effect of HRT on plasma lipids in diabetic postmenopausal women. Baseline serum PON1 arylesterase activity was significantly ($P < .001$) lower in the postmenopausal diabetic women (149 ± 38 μmol/mL/min; n = 47) than values in healthy postmenopausal women (173 ± 32 μmol/mL/min; n = 51). Serum PON1 activity increased (10%) significantly ($P = .009$) in diabetic women treated with HRT compared with placebo. A significant ($P = .02$) interaction between baseline PON1 activity and treatment indicated a greater increase in PON1 activity during HRT in women with lower baseline activities. At baseline, serum PON1 arylesterase activity was correlated significantly with plasma HDL cholesterol levels in diabetic women ($r = 0.333$, $P = .01$, n = 47), and the increase in serum PON1 activity was correlated significantly with the change in plasma HDL cholesterol during HRT ($r = 0.659$, $P = .0001$, n = 28). These data suggest that serum PON1 activity is abnormally low in postmenopausal women with type 2 diabetes and increases during HRT, particularly in women with lower baseline levels and in those who show a concomitant increase in HDL cholesterol.

PARAOXONASE (PON1) is an esterase associated with a high-density lipoprotein (HDL) particle containing clusterin and apolipoprotein A-I (apoA-I) in human serum.[1] The enzyme hydrolyses arylesters such as phenylacetate and organophosphates such as paraoxon.[1] Serum PON1 also hydrolyzes proinflammatory oxidized lipids present in oxidized low-density lipoproteins (LDL) and destroys their potentially atherogenic properties.[2] In addition, PON1 hydrolyzes lipid peroxides in atherosclerotic lesions,[3] where they promote progression of atherogenesis.[4] There is evidence that low PON1 activity may be associated with an increased risk of arterial lesion formation. Mice lacking serum PON1 are abnormally susceptible to diet-induced atherosclerosis.[5] Also, serum PON1 paraoxonase/arylesterase activities are low in survivors of myocardial infarction[6] and in subjects at increased risk of coronary artery disease (CAD), including those with hypercholesterolemia[7] and type 2 diabetes.[8-10]

The altered hormonal state after menopause may represent an additional cardiovascular risk factor in women with type 2 diabetes. Estrogen-replacement therapy improves lipid risk factors for CAD by decreasing plasma LDL cholesterol and apolipoprotein B (apoB) levels and increasing HDL cholesterol and apoA-I levels in postmenopausal diabetic women.[11] In women with an intact uterus, a progestin is added to estrogen therapy to prevent excess risk of endometrial cancer. In nondiabetic postmenopausal women, combined hormone-replacement therapy (HRT) with a conjugated equine estrogen and medroxyprogesterone acetate increases plasma HDL cholesterol levels.[12] Whether HRT increases serum PON1 activity in postmenopausal diabetic women is unclear. The present study aimed to test the effect of HRT with a conjugated equine estrogen and medroxyprogesterone acetate on serum PON1 arylesterase activity during the final phase of a randomized cross-over trial of HRT on plasma lipids, lipoproteins, and apolipoproteins in postmenopausal women with type 2 diabetes. Arylesterase activity of PON1 was measured because it is markedly greater and is therefore determined with greater precision than the corresponding activity with paraoxon as the substrate.

PATIENTS AND METHODS

Patients

Participants were recruited among postmenopausal women with type 2 diabetes who attended the diabetes department of Dunedin Hospital. Postmenopausal was defined as absence of menstrual periods for more than 2 years. Women were excluded if they had poorly controlled diabetes (glycated hemoglobin [HBA_{1c}] > 10%); a concomitant significant medical disorder; contraindications to HRT, including a history of breast or endometrial cancer; undiagnosed vaginal bleeding; uncontrolled hypertension; or severe liver dysfunction or they met the current national criteria for lipid-lowering therapy with statins. Each subject had a detailed medical history and examination performed by one investigator (P.J.M.). Cardiovascular disease was present in 14% of the diabetic women. Healthy postmenopausal women (n = 51) were also recruited from the local population through advertisement in the newspaper.

Study Design

Serum PON1 activity was measured during the second arm after an 8-week washout period of a randomized, placebo-controlled, crossover

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study designed to test the effect of HRT on plasma concentrations of lipids, lipoproteins, and apolipoproteins in postmenopausal diabetic women. Thus, the present study has a randomized, placebo-controlled, parallel design. Serum samples from the first arm of the study suitable for serum PON1 measurement were not available. Before the first arm of the study, women who met the selection criteria were individually randomized in permuted blocks of four subjects to receive either placebo or HRT. Permuted blocks (n = 6) were selected using random numbers generated by a computer. The randomization and allocation of subjects to treatment were performed by one investigator (S.J.) who did not interact with the patients at any other time during the study. Measurements relevant to the present study were made at baseline (end of the washout period) and 6 months later, at the end of the study. The staff who performed the biochemical analyses were blinded to the treatment received by the patients. Study variables were also measured in the healthy postmenopausal women.

Treatment

HRT consisted of conjugated equine estrogen (Premarin, 0.625 mg; Wyeth-Ayerst Laboratories, Philadelphia, PA) and medroxyprogesterone acetate (Provera, 2.5 mg; Wyeth-Ayerst) combined in a single capsule identical to the placebo capsule. To minimize acute side effects of HRT, study medication was titrated upward over a 4-week period. At the end of this time, diabetic women were receiving 1 capsule per day of either placebo or HRT. Patients were seen at 3-month intervals to check for adverse events, analyze compliance by capsule counting, record body weight, and measure blood lipids. Compliance with the study medication was defined as tablet count >80%. Patients were withdrawn from the study if they developed a serious adverse reaction to study medication, suffered a serious concurrent illness contraindicating HRT, or began receiving lipid-lowering therapy. Recruitment of patients ended in 1996. Subjects gave written informed consent, and the study was approved by the ethics committee of the Southern Regional Health Authority.

Laboratory Methods

Fasted venous blood was collected in tubes containing EDTA or potassium fluoride or in plain tubes. Plasma and serum were immediately separated by low-speed centrifugation at 4°C. All measurements except lipoprotein separations were made in aliquots of plasma and serum stored at −80°C. Serum PON1 arylesterase activity was measured as previously described.[13] Briefly, serum diluted in Tris buffer (20 mmol/L, pH 7.4) was added to 2 mL of phenylacetate solution (1 mmol/L in Tris buffer, pH 8), and the increase in absorbance at 270 nm was monitored at 1-minute intervals during the first 3 minutes. Blanks to correct for spontaneous hydrolysis of phenylacetate were included. The increase in absorbance was essentially linear during the 3-minute period, and using the molar extinction coefficient 1,310 $(mol/L)^{-1}$ cm^{-1}, the arylesterase activity was calculated. The intra-assay coefficient of variation for the assay was 5%, and the interassay coefficient of variation for measurements of pooled serum stored at −80°C was 12%.

Plasma very–low-density lipoproteins (VLDL) were separated by ultracentrifugation of EDTA plasma according to the Lipid Research Clinics' protocol.[14] HDL cholesterol was measured in the supernatant after precipitation of apoB-containing lipoproteins with dextran sulphate and magnesium chloride.[15] Cholesterol and triglycerides were measured in plasma and plasma fractions using commercial enzymatic kits (Boehringer Mannheim, Mannheim, Germany). Plasma lipid measurements were under good quality control (coefficient of variation routinely <3%). Plasma LDL cholesterol was calculated by subtracting HDL cholesterol from the cholesterol in the d > 1.006 g/mL plasma fraction. Serum apoA-I and apoB were measured by immunoturbidimetry.[16] Plasma glucose was measured enzymatically by automated methods using a commercial kit (Boehringer Mannheim). Hemoglobin A_{1c} (HBA_{1c}) was measured using a commercial kit (Glycotest, Pierce, Rockford, IL).

Statistics

Values are expressed as means ± SD unless otherwise stated. Multivariate linear regression analysis with final values as the dependent variable and baseline values and treatment group as independent variables with an interaction term of baseline values with treatment was used to test for differences between HRT and placebo treatments in the data.[17] When a significant difference between HRT and placebo treatments was detected, a paired *t* test was used to estimate the treatment effect within the HRT group. Student's *t* test was used to compare mean values between diabetic patients and healthy subjects. Analysis of covariance was used to compare baseline levels of serum PON1 activity between diabetic women and healthy women, controlling for age or plasma HDL cholesterol levels. Pearson's product-moment correlation coefficient was used to test for relationships between variables. Two-tailed tests of significance were used, and a *P* value of <.05 was considered statistically significant.

RESULTS

Seventy diabetic patients were screened and 61 met the entry criteria for the study. Participants were randomized to receive either HRT (n = 29) or placebo (n = 32) for the first 6-month arm of the study. This period was not part of the present investigation. After the washout period, 28 subjects (mean age 65 ± 7 years) who switched to HRT and 19 subjects (mean age 61 ± 8 years) who switched to placebo completed the study. Nine subjects withdrew while receiving HRT because of adverse effects (n = 4), cardiovascular event (n = 1), personal reasons (n = 2), and commencement of lipid-lowering therapy (n = 2). Two subjects withdrew while receiving placebo because of cancer of the bowel (n = 1) and a cerebrovascular event (n = 1). Two subjects were withdrawn during the washout period as a result of a cardiovascular event (n = 1) and peripheral vascular event (n = 1), and both subjects had received placebo during the first arm of the study. Forty-eight subjects completed the study, and all of these were compliant with the study medication (tablet count > 80%). Serum PON1 activity was not measured in one patient because of a lack of suitable samples. Altogether, results from 47 diabetic subjects were available. Five women currently smoked cigarettes (placebo, n = 2; HRT, n = 3), 6 were treated with diet alone (placebo, n = 1; HRT, n = 5), 22 were receiving oral hypoglycemic agents (placebo, n = 10; HRT, n = 12), 19 were treated with insulin (placebo, n = 8; HRT, n = 11), and 26 were taking angiotensin-converting enzyme (ACE) inhibitors (placebo, n = 13; HRT, n = 13). One subject (placebo) was taking multivitamin tablets, and one (HRT) was taking vitamin E capsules. None was receiving lipid-lowering therapy.

Table 1 shows serum PON1 arylesterase activity, anthropometric data, HBA_{1c}, fasting plasma glucose, lipid, lipoprotein, and apolipoprotein concentrations in the 47 postmenopausal women with diabetes and 51 healthy postmenopausal women. Serum PON1 arylesterase activity was significantly lower in diabetic women compared with healthy women. The diabetic women were younger and, as expected, their levels of plasma triglycerides and VLDL lipids and body mass index (BMI) were significantly higher, and their levels of HDL cholesterol

Table 1. Serum PON1 Arylesterase Activity and Other Characteristics of Diabetic and Healthy Postmenopausal Women

	Diabetic Women (n = 47)	Healthy Women (n = 51)	*P*
PON1 (μmol/mL/min)	149 ± 38	173 ± 32	<.001
Age (yr)	64 ± 8	67 ± 7	<.001
BMI (kg/m^2)	32.3 ± 5.7	27.3 ± 6.3	.008
HBA_{1c} (%)	7.5 ± 1.9	ND	
Fasting glucose (mmol/L)	10.2 ± 3.9	ND	
TC (mmol/L)	6.58 ± 1.23	7.00 ± 1.23	.04
VLDL-C (mmol/L)	0.95 ± 0.75	0.68 ± 0.53	.01
LDL-C (mmol/L)	4.34 ± 1.08	4.82 ± 1.06	.01
HDL-C (mmol/L)	1.16 ± 0.25	1.44 ± 0.36	<.001
TG (mmol/L)	2.69 ± 1.39	2.02 ± 0.95	.008
VLDL-TG (mmol/L)	1.93 ± 1.25	1.26 ± 0.79	.002
ApoA-I (g/L)	1.39 ± 0.22	1.52 ± 0.25	.01
ApoB (g/L)	1.14 ± 0.32	1.24 ± 0.28	.07

NOTE. Values are means ± SD.

Abbreviations: TC, total cholesterol; VLDL-C, VLDL cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglyceride; VLDL-TG, VLDL triglyceride; ND, not determined.

and apoA-I were significantly lower than those in healthy women. Plasma cholesterol and LDL cholesterol levels were significantly higher in the healthy controls. The difference in serum PON1 activity between diabetic women and healthy controls remained significant when data were adjusted for age ($P < .001$) or plasma HDL cholesterol levels ($P = .02$) by analysis of covariance.

Table 2 shows serum PON1 activity, anthropometric data, HBA_{1c}, and fasting plasma glucose, lipid, lipoprotein, and apolipoprotein concentrations at baseline and at the end of 6 months of treatment with HRT or placebo in diabetic, postmenopausal women. Baseline means of study variables were comparable between postmenopausal diabetic women assigned to HRT or placebo, except for body weight, which was 13% lower, and serum apoB levels, which were 14% higher in the HRT group. Multivariate regression of final values on baseline values indicated that there was a significant increase in serum PON1 activity and a significant decrease in plasma VLDL cholesterol levels compared with placebo. The changes in these variables in the HRT group during the study were also significant. There were significant interactions between treatment and baseline levels of plasma VLDL cholesterol ($P = .0001$) and serum PON1 activity ($P = .02$) in the regression models. Regression models of the effect of HRT compared with placebo treatment on PON1 activity are summarized in Table 3. The significant interaction between treatment and baseline PON1 activity indicates that the magnitude of the treatment effect of HRT compared with placebo depends on baseline PON1 activity. The regression equation in model 1 indicates that at a baseline PON1 activity of 70 μmol/mL/min, the mean difference in activity between HRT and placebo treatments is 51 μmol/mL/min and decreases to 0 at a baseline activity of approximately 170 μmol/mL/min. This range of baseline values (70 to 170 μmol/mL/min) includes 77% of serum PON1 activities in the group of diabetic women. When smoking status and treatments with insulin and angiotensin-converting enzyme (ACE) inhibitor were added to the independent variables in model 1 (model 2; Table 3), both the effect of treatment with HRT compared with placebo and treatment × baseline PON1 interaction remained significant. In this regression model, smoking status and insulin therapy were significant predictors of final serum PON1 activity. This difference between treatments remained significant ($P = .002$) when therapy with oral hypoglycemic agents was included in the regression model. The effect of treatment with HRT compared with placebo on serum PON1 activity also remained significantly different ($P = .006$) when baseline BMI and serum apoB levels were added to independent variables in model 1 (Table 3). At the end of the study, mean serum PON1 arylesterase activity in women assigned to HRT was not significantly different ($P = .31$), whereas the corresponding activity in women assigned to pla-

Table 2. Serum PON1 activity, BMI, HBA_{1c}, Fasting Plasma Glucose, and Lipid, Lipoprotein, and Apolipoprotein Concentrations in Diabetic Postmenopausal Women at Baseline and After 6 Months Treatment With HRT or Placebo

	Placebo (n = 19)		HRT (n = 28)	
	Baseline	6 mo	Baseline	6 mo
PON1 (μmol/mL/min)	148 ± 36	152 ± 46	150 ± 39	165 ± 35*
BMI (kg/m^2)	34.9 ± 5.8	35.9 ± 6.1	30.8 ± 5.1	30.0 ± 5.5
HBA_{1c} (%)	7.8 ± 2.3	8.5 ± 2.1	7.3 ± 1.6	7.9 ± 1.6
Glucose (mmol/L)	10.66 ± 4.69	10.38 ± 4.1	9.97 ± 3.30	8.37 ± 2.1
TC (mmol/L)	6.31 ± 1.43	5.84 ± 1.49	6.76 ± 1.05	6.12 ± 1.12
VLDL-C (mmol/L)	0.84 ± 0.55	0.86 ± 0.79	1.03 ± 0.85	0.85 ± 0.59†
LDL-C (mmol/L)	4.21 ± 1.09	3.82 ± 0.88	4.43 ± 1.08	3.93 ± 1.00
HDL-C (mmol/L)	1.15 ± 0.27	1.18 ± 0.42	1.18 ± 0.24	1.22 ± 0.27
TG (mmol/L)	2.57 ± 1.39	2.47 ± 1.38	2.77 ± 1.41	2.62 ± 1.16
VLDL-TG (mmol/L)	1.85 ± 1.13	1.78 ± 1.30	1.98 ± 1.35	1.79 ± 1.08
apoA-I (g/L)	1.38 ± 0.21	1.40 ± 0.23	1.40 ± 0.23	1.50 ± 0.21
apoB (g/L)	1.05 ± 0.34	1.17 ± 0.31	1.20 ± 0.29	1.23 ± 0.26

NOTE. Values are means ± SD.

* Significant effect of HRT compared with placebo at $P = .009$ and significant ($P = .01$) within-group change during HRT.

† Significant effect of HRT compared with placebo at $P = .01$ and significant ($P = .04$) within-group change during HRT.

Table 3. Regression Models of the Effect of Treatment With HRT or Placebo on Serum PON1 Arylesterase Activity in Postmenopausal Diabetic Women

Independent Variables	Coefficient	95% Confidence Interval	*P*
Model 1			
Constant	−12.1		
Treatment (0,1)	85.9	22.1 to 149.7	.009
Baseline PON1 (μmol/mL/min)	1.1	0.8 to 1.5	.0001
Treatment × baseline PON1	−0.5	−0.9 to −0.1	.02
Model 2			
Constant	−19.6		
Treatment (0,1)	94.5	36.7 to 152.2	.002
Baseline PON1 (μmol/mL/min)	1.2	0.9 to 1.5	.0001
Treatment × baseline PON1	−0.6	−0.9 to −0.2	.004
Insulin therapy (0,1)	21.5	7.6 to 35.4	.003
Smoking (0,1)	31.1	8.4 to 53.8	.008
ACE-I (0,1)	−7.4	−22.0 to 7.3	.31

NOTE. Dependent variable: final PON1 (μmol/mL/min).

cebo remained significantly ($P = .04$) lower than mean rates in healthy postmenopausal women.

At baseline, serum PON1 arylesterase activity was correlated significantly with plasma HDL cholesterol ($r = 0.333$, $P = .01$), LDL cholesterol ($r = 0.295$, $P = .022$), and serum apoA-I ($r = 0.250$, $P = .045$) in diabetic women (n = 47). The increase in serum PON1 arylesterase activity correlated significantly with change in HDL cholesterol in these women during HRT (Fig 1).

DISCUSSION

Our data indicate that serum PON1 arylesterase activity is abnormally low and increases during 6 months of HRT in diabetic postmenopausal women, particularly in those with lower baseline levels and those who show an increase in HDL cholesterol levels with HRT. It is conceivable that an increase in serum PON1 arylesterase activity may decrease the risk of lipoprotein oxidative modification to potentially atherogenic particles and reduce levels of damaging lipid peroxides in atherosclerotic lesions.

Abnormally low serum PON1 arylesterase activity suggests that PON1 concentrations are low in postmenopausal diabetic women. A previous study has reported that arylesterase activity reflects serum PON1 concentration.[18] Low serum PON1 arylesterase activity in diabetic postmenopausal women is in keeping with reported low rates of PON1 paraoxonase activity in serum from patients with type 2 diabetes.[8-10] Factors mainly responsible for low serum PON1 activity in diabetic patients remain uncertain. Abnormal composition of diabetic HDL and glycation of PON1 have been suggested as possible causes of this low activity.[8] Glycation of HDL in vitro decreases PON1 activity by 65%.[19] Low plasma HDL levels did not account for low serum PON1 activity in diabetic patients compared with healthy women in the present study or in a previous report.[8] Variation in the PON1 gene affects PON1 paraoxonase activity and arylesterase activity to a lesser extent but is not responsible for the lower PON1 activity in type 2 diabetes.[8]

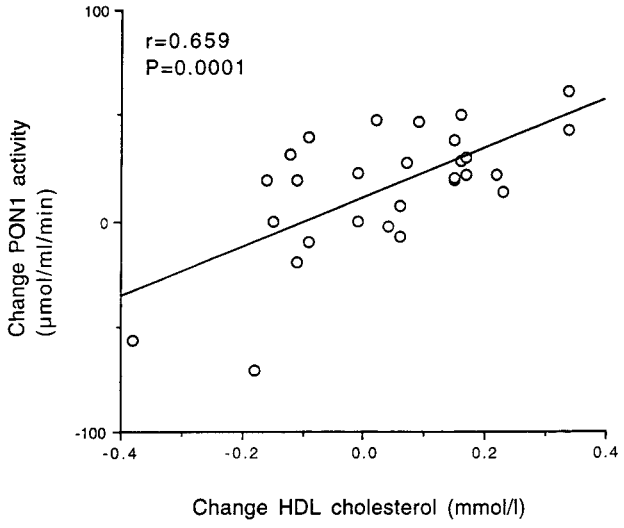

Fig 1. Relationship between change in PON1 activity and change in HDL cholesterol level during HRT (n = 28).

Our data suggest that 6 months of HRT increases serum PON1 arylesterase activity in postmenopausal diabetic women, and the magnitude of this increase may depend on baseline PON1 activity and the response of HDL cholesterol to HRT. Women with lower PON1 activity and those whose HDL cholesterol levels increased experienced a greater increase in PON1 activity during HRT. Factors responsible for this interaction between baseline PON1 activity and HRT are unclear. Smoking and treatments with ACE inhibitors, insulin, or oral hypoglycemic agents did not appear to affect the interaction or the HRT-induced increase in serum PON1 activity. Smoking has been associated with reduced serum PON1 activity in a previous study.[20] It is possible that genetic variation in PON1 that influences serum PON1 arylesterase/paraoxonase activity and concentration[18,20] also influences the magnitude of the response of PON1 arylesterase activity to HRT. An increase in serum PON1 arylesterase activity may reflect an increase in circulating PON1 concentration.[18] Thus, paraoxonase, esterase, and peroxidase activities associated with PON1[3] may also increase in diabetic women during HRT. These peroxidase and esterase activities apparently hydrolyze lipid peroxides in human atherosclerotic lesions.[3]

The association between serum PON1 arylesterase activity and HDL cholesterol at baseline in postmenopausal diabetic women appears to be in keeping with the reported association between these variables in normal volunteers.[21] These relationships may be attributable to the fact that PON1 is carried mainly by an HDL subfraction in blood. On the other hand, there was no evidence of a similar relationship in the healthy postmenopausal women we studied. Because many HDL particles do not contain PON1, bulk HDL levels can presumably change without necessarily affecting PON1 levels. The relationship between the increase in serum PON1 arylesterase activity and the change in plasma HDL cholesterol level in postmenopausal diabetic women during HRT is in accord with the relationship between serum PON1 arylesterase activity and plasma HDL cholesterol level at baseline. The increase in HDL cholesterol in some but not other postmenopausal diabetic women treated with conjugated equine estrogen and medroxyprogesterone acetate may not be attributable merely to random variation. A large randomized, placebo-controlled study has reported a small increase in plasma HDL cholesterol levels in healthy women receiving this type of HRT.[12] In the present study, a mean increase in HDL cholesterol of a similar magnitude was recorded but was not statistically significant, possibly because of the smaller numbers of participants. It is possible that variation in genes that regulate lipoprotein metabolism contribute to individual variation in response of plasma HDL cholesterol to HRT.

Therapies that increase plasma HDL cholesterol levels do not inevitably increase serum PON1 activity. Durrington et al[22] have reported unchanged serum PON1 paraoxonase activity in the face of increased plasma HDL cholesterol levels in patients with type IIb hyperlipoproteinemia treated with gemfibrozil or bezafibrate. It is conceivable that oral estrogen also increases hepatic synthesis of PON1 as well as apoA-I, but as yet no evidence is available to support this hypothesis. Oral estrogen

has an important effect on hepatic metabolism and apparently stimulates hepatic and intestinal synthesis of apoA-I.[23] Progestins can oppose the increase in HDL cholesterol induced by estrogen treatment and may also tend to oppose an associated increase in PON1 activity.

Treatment with conjugated equine estrogen and medroxyprogesterone acetate decreases plasma cholesterol and LDL cholesterol levels and increases HDL cholesterol levels in postmenopausal women.[12] In the present study, plasma cholesterol and LDL cholesterol levels decreased not only in women receiving HRT but also in those receiving placebo. The decrease in these variables in the placebo group did not appear to be a continued effect of previous HRT treatment. In the placebo group, the mean plasma cholesterol level at the end of the previous HRT treatment was 5.72 mmol/L and increased during the 8-week washout period to 6.31 mmol/L, which is consistent with the cessation of HRT. Whether there was a cholesterol-lowering change in habitual diet in women during the placebo period is uncertain. The decrease in VLDL cholesterol during HRT must be viewed with caution. This decrease may reflect regression to the mean because levels were higher in women at the start of HRT than in those receiving placebo. Also, few if any previous studies have reported a decrease in plasma VLDL cholesterol during oral HRT. When data from both arms of the present cross-over trial were analyzed, plasma cholesterol and LDL cholesterol levels at the end of HRT were clearly lower, and HDL cholesterol and VLDL cholesterol levels were not clearly different from levels at the end of placebo treatment (submitted for publication).

This study has limitations. It was commenced during a randomized cross-over trial designed to test the effect of HRT on blood lipid levels. The loss of several patients all from one treatment group before baseline measurements at the end of the washout period may have introduced bias. Imbalances in BMI and serum apoB between the HRT and placebo groups at baseline did not influence the main finding of increased serum PON1 arylesterase activity during HRT. Nevertheless, it is possible that the loss of subjects from the study introduced differences in unmeasured variables between the placebo and HRT groups that may have influenced the findings. Previous HRT (followed by 8 weeks washout) did not appear to affect serum PON1 activity, which remained unchanged in women who received placebo treatment during the study. We cannot exclude the possibility that change in serum butyrylesterase activity, which is elevated in diabetic patients,[24] accounts for part of the change in arylesterase activity during HRT in the present study. On the other hand, we calculate that butyrylesterase activity may contribute approximately 5% to arylesterase activity in diabetic women, and this level of activity is similar to the error in the measurement of PON1 activity. The study was of relatively short duration, and our findings need to be confirmed in a longer trial.

In conclusion, the present study suggests that HRT with a conjugated equine estrogen and medroxyprogesterone acetate may increase serum PON1 arylesterase activity in postmenopausal diabetic women, with the greatest increase in women with lower baseline levels and in those who experience an increase in HDL cholesterol levels. The magnitude of this HRT-induced increase in serum PON1 arylesterase activity appears to be clinically relevant because the activity in diabetic postmenopausal women during treatment nearly reached the higher levels in their healthy counterparts. Increased PON1 activity in diabetic women with the lowest activity may reduce the risk of vascular complications. Low serum PON1 activity has been previously associated with increased risk of diabetic vascular complications.[10] PON1 hydrolyzes lipid peroxides in lipoproteins and atherosclerotic lesions,[2,3] where they may have atherogenic properties. Thus, it is conceivable that an HRT-induced increase in serum PON1 activity is associated with a delay in the formation and progression of atherosclerotic disease in postmenopausal diabetic women.

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