Hormone Replacement Therapy Enhances Postprandial Lipid Metabolism in Postmenopausal Women

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Postmenopausal estrogen therapy reduces cardiovascular morbidity and mortality, except in women with advanced coronary disease. This beneficial effect is partly attributed to a reduction of fasting plasma total and low-density lipoprotein cholesterol (LDL-C) and an elevation of plasma high-density lipoprotein cholesterol (HDL-C) concentrations. Since postprandial lipemia seems to play a role in the pathogenesis of coronary artery disease, we evaluated the effect of hormone replacement therapy (HRT) on postprandial lipoprotein metabolism in 14 normolipemic postmenopausal women. A vitamin A fat-loading test before and after three cycles of treatment with a sequential combination of conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA) was used to label chylomicrons and chylomicron remnants with retinyl palmitate (RP), and RP clearance was assessed over an 8-hour period postprandially. Following 3 months of HRT, fasting total cholesterol and LDL-C levels were reduced 9.8% ($P = .049$) and 16.5% ($P = .023$), respectively. Fasting HDL-C levels increased 18.9% ($P = .001$). Fasting triglycerides (TGs) increased, but not significantly. Postprandial integrated plasma TGs did not change significantly. The integrated RP levels in whole plasma and chylomicron (Svedberg flotation units [S_f] > 1,000) and nonchylomicron ($S_f < 1,000$) fractions were reduced 58% ($P = .043$), 78% ($P = .041$), and 75% ($P = .001$), respectively, after hormonal treatment. Enhanced clearance of chylomicrons and chylomicron remnants by HRT may contribute to the protective effect of estrogens against cardiovascular disease in normolipemic postmenopausal women.

POSTMENOPAUSAL ESTROGEN therapy reduces cardiovascular morbidity and mortality,[1] except in women with advanced coronary disease.[2] These beneficial effects are partly attributed to a reduction of fasting low-density lipoprotein cholesterol (LDL-C) and an elevation of high-density lipoprotein cholesterol (HDL-C) levels.[3] Recently, based on observations in a small group of women, Westerveld et al[4] suggested that exogenous estrogens also may improve postprandial lipid metabolism, which may be an additional mechanism underlying the protective effect of hormone replacement therapy (HRT) on cardiovascular disease in women.

Most of our lives are spent in the postprandial state, during which time the vessel wall is exposed to intestinally derived triglyceride (TG)-rich lipoproteins, ie, chylomicrons and chylomicron remnants. Based on animal and human studies, it has been suggested that these lipoproteins may be atherogenic since they are metabolized on the endothelial surface of arteries and their cholesterol becomes incorporated into the artery wall, where it may stimulate formation of atherosclerotic lesions.[5-10]

Postprandial lipoprotein catabolism is believed to occur in two steps.[11] Initially, chylomicrons produced in the intestines that carry exogenous lipids interact with lipoprotein lipase in extrahepatic tissues. This results in TG hydrolysis and delivery of free fatty acids to the tissues. After most of the TGs are hydrolyzed, remnant particles are formed that are removed from the circulation by hepatocyte receptors that recognize apolipoprotein E.

The role of chylomicrons in the development of atherosclerosis is not clear, while the role of chylomicron remnants is documented in many animal and human studies.[12] In the study by Westerveld et al,[4] it was shown that HRT significantly reduced chylomicron levels only. A tendency for a reduction in the level of chylomicron remnants was also demonstrated but was not significant. However, the study was performed on a small group of six patients. It is important to evaluate the exact effect of HRT on the levels of the various postprandial lipoproteins, due to their different atherogenicity.

The objective of the present study was to examine the effect of estrogen therapy on chylomicrons and chylomicron remnants in a larger group of women. We used the vitamin A fat-loading test, which specifically labels chylomicrons and chylomicron remnants with retinyl palmitate (RP) and was found to be efficient for evaluating their metabolism.

SUBJECTS AND METHODS

Subjects

Fourteen women in natural menopause for at least 2 years were enrolled in the study. Patients were included in the study if they met the following criteria: good general health, follicle-stimulating hormone and luteinizing hormone blood levels greater than 40 and 30 mIU/mL, respectively, estradiol concentration less than 18 nmol/L, body mass index less than 25 kg/m^2, normal liver, renal, and thyroid function, and normal fasting and glucose tolerance tests. The study protocol was approved by the Tel Aviv Medical Center Ethics Committee, and all women provided informed consent.

Study Design

The study consisted of a baseline phase and a treatment phase. Patients were instructed to maintain their regular diet, routine physical activity, and smoking habit throughout the study period. No medications influencing plasma lipids were permitted.

In the baseline phase, three blood samples were drawn for fasting lipid and lipoprotein determinations, and participants underwent a vitamin A fat-loading test. HRT was then started with a cyclic combination containing conjugated equine estrogen (CEE) 1.25 mg daily for 13 days and combined medroxyprogesterone acetate (MPA) 5 mg plus CEE for another 12 days. No hormones were taken for the subsequent 5 days, and menses appeared in all women. Three months later, blood was again drawn for determination of fasting lipids and lipoproteins, and the vitamin A fat-loading test was repeated.

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Vitamin A Fat-Loading Test

The vitamin A fat-loading test was performed as previously described.[13] Briefly, after an overnight 12-hour fast, subjects were given a fatty meal plus 60,000 U aqueous vitamin A/m^2 body surface area. The fatty meal contained 50 g fat/m^2 body surface area and consisted of 65% calories as fat, 20% as carbohydrate, and 15% as protein. It contained 600 mg cholesterol/1,000 cal. The polyunsaturated to saturated fat ratio was 0.3. The meal was given as a milkshake, scrambled eggs, bread, and cheese, and was eaten within 10 minutes. Vitamin A was added to the milkshake. After the meal, the subjects fasted for 8 hours except for water intake, which was permitted ad libitum. To measure the level of RP, blood samples were drawn before the meal and every hour thereafter for 8 hours. All participants tolerated the meal well, and none had diarrhea or other symptoms of malabsorption.

Analysis of Samples

Venous blood was drawn from the forearm and transferred to a tube containing sodium EDTA. Samples were immediately centrifuged at 1,500 × *g* for 15 minutes, and 1 mL plasma was stored wrapped in foil at −20°C for the retinyl ester assay. An aliquot of 2.5 mL plasma was transferred to a .50 × 2-in cellulose nitrate tube and overlayered with 2.5 mL sodium chloride solution (d = 1.006 g/mL). The tubes were subjected to preparative ultracentrifugation at 1.6 × 10^6 *g*/min in a Beckman SW-55 rotor (Beckman Instruments, Fullerton, CA) to float chylomicron particles with S_f greater than 1,000. The chylomicron-containing supernatant was removed and brought to a total volume of 2 mL with saline. The infranatant was brought to a volume of 5 mL with saline. Aliquots of supernatant and infranatant (0.5 mL) were wrapped in foil and assayed for retinyl ester. This procedure separates a predominantly chylomicron population from a predominantly chylomicron remnant population.[13,14]

Retinyl Ester Assay

The assays were performed in subdued light with high-performance liquid chromatography (HPLC)-grade solvents. Retinyl acetate was added to the samples as an internal standard. The samples were then mixed with 4 mL ethanol, 5 mL hexane, and 4 mL water, with vortexing between each addition. Two phases were formed, and 4 mL of the upper (hexane) phase was removed and evaporated under nitrogen.[15] The residue was dissolved in a small volume of benzene, and an aliquot was injected into a 5-μm HPLC ODS-18 radial compression column; 100% methanol was used as the mobile phase at a flow rate of 2 mL/min. The effluent was monitored at 340 nm, and the RP peak was identified by comparison to the retention time of purified standard (Sigma Chemical, St Louis, MO). In agreement with previous reports,[14] it was found that 75% to 85% of total plasma retinyl esters were accounted for by RP. In addition, the distribution of retinyl esters remained constant throughout the study. RP in plasma and lipoprotein fractions was quantified by the area ratio method[16] using retinyl acetate as a reference.[17] The efficiency of extraction of retinyl esters was more than 95%, and the variance of triplicate assays was less than 5.4% of the mean.

Lipid and Lipoprotein Determinations

Cholesterol and TG levels were measured enzymatically using the reagents cholesterol 236991 and triglyceride 126012 (Boehringer Mannheim, Indianapolis, IN). HDL-C was determined after precipitation of whole plasma with dextran sulfate–magnesium.

Apolipoprotein E Isoform Determination

DNA was extracted from white blood cells. The polymerase chain reaction was performed according to the method described by Wenham and Price.[18]

Statistical Analysis

Values are reported as the mean ± SD. Differences in the body mass index and fasting lipids and lipoproteins before and during HRT were analyzed for significance using the paired *t* test. A *P* level less than .05 was considered significant.

Evaluation of the significance between the mean area under the curve (AUC) for RP in whole plasma and chylomicron and nonchylomicron fractions was performed using a *t* test for paired measurements. The relation between fasting lipids and lipoproteins and the mean values for the RP AUC in whole plasma and chylomicron and nonchylomicron fractions was assessed using the Pearson correlation test.

RESULTS

Salient characteristics before and during HRT for the 14 study participants are shown in Table 1. None of the participants were positive for the E2 allele of apolipoprotein E. The body mass index of the study participants did not change during treatment compared with pretreatment values. During treatment, the mean fasting total cholesterol decreased 9.8% (P = .049) and mean fasting LDL-C decreased 16.5% (P = .023). Mean fasting HDL-C levels increased 18.9% (P = .001) following 3 months of HRT. Fasting TG showed a trend to increase, which did not reach statistical significance.

The postprandial whole-plasma RP 8-hour AUC, as well as the chylomicron ($S_f > 1{,}000$) and nonchylomicron ($S_f < 1{,}000$) RP 8-hour AUCs, decreased significantly by a mean of 58% (P = .043), 78% (P = .041), and 75% (P = .001), respectively, during HRT (Table 2 and Fig 1). The total postprandial TG 8-hour AUC was not changed during HRT.

Positive correlations were found between the mean decrement in chylomicron remnant RP levels and between the mean decrement in fasting total cholesterol and LDL-C levels during treatment (r = .53, P = .025 and r = .54, P = .025, respectively).

DISCUSSION

HRT significantly improved the postprandial elimination of chylomicron and chylomicron remnant RP, measured as the respective RP 8-hour AUCs, in normolipemic postmenopausal women. Westerveld et al[4] made similar observations in six postmenopausal women, although in their study no significant reduction was noted in the chylomicron remnant fraction. This discrepancy might be due to the sample size, as the mean AUCs in their population decreased more than 37% after 6 weeks of 17β-estradiol treatment but did not reach statistical significance. Similar findings were reported in normolipemic premenopausal women receiving oral contraceptives.[19]

Elevated plasma remnant concentrations have been identified as a risk indicator for coronary artery disease in both postmenopausal women[20] and individuals with type III hyperlipoprotein-

Table 1. Fasting Lipids and Lipoproteins in Postmenopausal Women Before and During HRT

Parameter	Before HRT	During HRT	*P*
Total cholesterol	6.32 ± 1.14	5.67 ± 0.67	.049
TG	1.24 ± 0.58	1.35 ± 0.47	.3
LDL-C	4.22 ± 0.98	3.52 ± 0.57	.023
HDL-C	1.50 ± 0.15	1.63 ± 0.20	.001

NOTE. Values are the mean ± SD (mmol/L).

Table 2. Postprandial TG 8-Hour AUC and RP 8-Hour AUC in the Total Plasma, Chylomicron Fraction ($S_f > 1,000$), and Nonchylomicron Fraction ($S_f < 1,000$) Before and During HRT in 14 Postmenopausal Women

Parameter	Before HRT	During HRT	P
Total plasma RP AUC ($\mu mol \cdot L^{-1} \cdot h$)	40.25 ± 23.03	25.32 ± 15.1	.043
Chylomicron RP AUC ($\mu mol \cdot L^{-1} \cdot h$)	26.08 ± 18.64	14.58 ± 10.53	.041
Nonchylomicron RP AUC ($\mu mol \cdot L^{-1} \cdot h$)	9.29 ± 4.57	5.29 ± 2.53	.001
Total plasma TG AUC ($mmol \cdot L^{-1} \cdot h$)	10 45 ± 5.25	9.52 ± 5.16	.35

NOTE. Values are the mean ± SD.

emia.[21,22] Therefore, the effect of HRT on these particles as shown by our results adds another mechanism to the beneficial effect on coronary artery disease in addition to the well-known effect on fasting lipoproteins. The exact mechanism underlying the improved elimination of chylomicron and chylomicron remnants is yet unclear. However, it has been speculated that an estrogen-induced increase in hepatic LDL (B/E) receptor synthesis upregulates their activity. Since these are common receptors for LDL and chylomicron remnants, it may account for the accelerated clearance of the last two.[23-26] The positive correlation between the posttreatment reduction of chylomicron remnant RP and the posttreatment reduction of fasting total cholesterol and LDL-C observed in our study supports this common pathway of clearance for LDL-C and chylomicron remnants. In this study, we chose a relatively high dose of conjugated estrogens, ie, 1.25 mg/d rather than 0.625 mg/d, to accentuate the expected beneficial effect of estrogens on the catabolism of postprandial lipoproteins. However, this is the dosage given most commonly to postmenopausal women in this country. A study using an estrogen dose of 0.625 mg/d would be of greater clinical relevance in other countries.

It is well known that interventions such as physical activity, weight reduction, or administration of fibric acid derivatives that decrease fasting TGs cause a concomitant increase in HDL-C[11]; however, estrogens cause an opposite effect. They elevate both fasting TG and HDL-C blood levels.[1] This particular mode of estrogen action is intriguing. Plasma TGs are found mainly in very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein and postprandially in chylomicrons and chylomicron remnants. The reciprocal relation between fasting plasma TG and HDL-C levels is in line with current concepts on the transfer of surface remnants from TG-rich lipoproteins to the HDL system.[11] In correspondence with this, our group and others have previously reported an enhanced clearance of postprandial lipoproteins in association with an elevation of HDL-C levels.[27,28] In this study, as expected, we showed that during estrogen treatment fasting HDL-C levels are elevated in the face of increased fasting TG levels, but more importantly, we demonstrated an enhanced catabolism of postprandial TG-rich lipoproteins.

Chylomicrons and VLDLs are thought to compete for the saturable lipoprotein lipase[29] such that the catabolic rate of chylomicrons is inversely related to the plasma level of VLDL. However, in this study, increased clearance of chylomicrons was not accompanied by a reduction of fasting TG levels. This is possibly the result of the estrogen-induced synthesis of large VLDL particles,[30,31] which are enriched with apolipoprotein E[32] and are therefore taken up more avidly by the liver.

In conclusion, HRT significantly enhanced the postprandial clearance of chylomicrons and chylomicron remnants in normolipemic postmenopausal women. It is also encouraging that the addition of progesterone to estrogen replacement does not abolish the beneficial effects on lipoprotein elimination that exert a protective effect against cardiovascular disease in women.

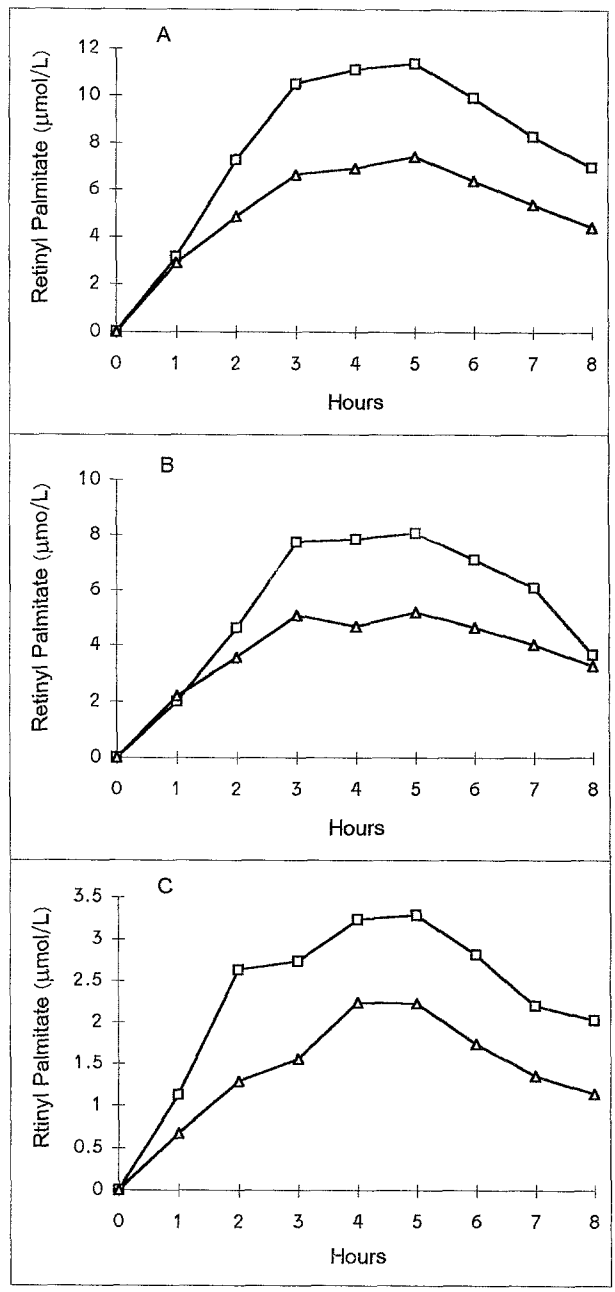

Fig 1. RP levels in the whole plasma (A), chylomicron fraction ($S_f > 1,000$, B), and nonchylomicron fraction ($S_f < 1,000$, C) 0-8 hours after a vitamin A-fat loading test before (□) and during (△) HRT in 14 postmenopausal women.

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