Enhancement of Cholesteryl Ester Transfer in Plasma by Hormone-Replacement Therapy

Andreas Ritsch, Susanne Kaser, Birgit Volgger, Elisabeth Abfalter, Wolfgang Sturm, Hannes Gänzer, Bernhard Föger, Rudolf Kirchmair, Christoph Ebenbichler, and Josef R. Patsch

To study possible mechanisms for the suggested protective effect of hormone-replacement therapy (HRT) with respect to cardiovascular disease we investigated lipoprotein parameters, mass and activity of lipoprotein-metabolizing enzymes, magnitude of postprandial lipemia, and vascular endothelial function in 13 postmenopausal women. All patients were examined before and 3 months after implementation of HRT with estrogen alone (group A, n = 6) or estrogen plus gestagen (group B, n = 7). HRT (groups A and B) resulted in enhanced total transfer of cholesteryl ester (CE) from high-density lipoprotein (HDL) to apolipoprotein B (apoB)-containing lipoproteins (56% ± 11.45% *v* 50.82% ± 13.68%, *P* < .05) and increased apoA-I plasma concentration (171 ± 30 *v* 147 ± 22 mg/dL, *P* < .05). Fasting triglycerides (TG) were increased (134 ± 40 *v* 115 ± 39 mg/dL, *P* < .05). In group A patients the magnitude of postprandial lipemia increased significantly (1,737 ± 756 *v* 1,475 ± 930 mg TG/dL plasma/8 h, *P* < .05) without any change in lipoprotein lipase (LPL) activity, but with a concomitant decrease in low-density lipoprotein (LDL) size. In both groups flow-mediated dilation (FMD) reflecting vascular endothelial function was not influenced, suggesting that HRT may not directly affect vascular function but rather alters lipoprotein metabolism. The increase of apoA-I was not accompanied by an equivalent rise of HDL cholesterol. Based on the present data this finding can be readily explained by an increase in CE transfer from HDL to TG-rich lipoproteins, which is not due to increased cholesteryl ester transfer protein (CETP) plasma levels, but rather reflects an increase in fasting and postprandial TG. In conclusion, the net effect of accelerated CE transfer due to HRT depends on the balance of proatherogenic aspects, like the generation of small dense LDL, and antiatherogenic aspects, like the stimulation of reverse cholesterol transport.

NUMEROUS OBSERVATIONAL studies have demonstrated beneficial effects of hormone-replacement therapy (HRT) with respect to cardiovascular disease in postmenopausal women.[1] This apparent benefit was attributed mostly to favorable effects on fasting lipid parameters, ie, decrease of total and low-density lipoprotein (LDL) cholesterol, as well as increase of high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I (apoA-I).[2,3] However, reports on the beneficial effects of HRT as regards cardiovascular disease were not confirmed in the only randomized controlled trial conducted to date, which had a neutral outcome.[4]

In terms of the risk factor role of blood lipids, the magnitude of postprandial lipemia in addition to fasting lipids has been shown to be associated with coronary artery disease.[5,6] Previous studies on postprandial lipemia in postmenopausal women showed an improved clearance of remnants from the circulation during alimentary lipemia after HRT.[7-9]

Most studies of postmenopausal women treated with HRT reported an increase in fasting triglycerides (TG).[3,10] The synchronous increase in both fasting HDL cholesterol and TG levels in plasma is somewhat odd in the face of the usual inverse relationship between the two.[11] This commonly inverse relationship may be partially explained by the action of cholesteryl ester transfer protein (CETP), as CE transfer in plasma increases with increased plasma concentrations of very-low-density lipoprotein (VLDL) TG.[12,13] Additionally, the postulated inverse relationship between plasma levels of CETP and HDL cholesterol is only found in hypertriglyceridemic patients.[14] The unusual constellation of high HDL cholesterol and high TG levels is most accentuated in CETP-deficient patients. We recently characterized the molecular defect in a patient with CETP deficiency, who had a drastically elevated HDL cholesterol level, together with highly elevated postprandial lipemia.[15] Hypertriglyceridemia has also been reported in CETP-deficient patients in Japan.[16] However, in this scenario the absence of CETP in the patients plasma is responsible for this lipid constellation, clearly demonstrating the role of CETP as a mediator between pools of TG and CE. Accordingly, changes in plasma concentration and activity of the lipid transfer proteins CETP or phospholipid transfer protein (PLTP) may be responsible, at least in part, for the unusual lipid profile in postmenopausal women treated with HRT. In previous studies, CETP concentration was not altered by HRT in postmenopausal women by HRT,[17] and no changes were found on plasma newly synthesized CE transfer.[18] This assay reflects both lecithin cholesterol acyltransferase (LCAT) and CETP activity, respectively, and is closely correlated with net CE mass transfer from HDL to apoB-containing lipoproteins.[19] To further investigate this issue, we set out to measure several parameters of lipid transfer proteins, including CETP mass and activity, CE transfer, and PLTP activity in postmenopausal women before and after treatment with estrogen or a combination of estrogen and gestagen. Our goal was to further understand the mechanism underlying the changes in plasma lipoprotein profiles and, possibly, that underlying the apparent protective effect in this clinical setting. For the latter purpose, we measured flow-mediated dilation (FMD), which directly

From the Department of Medicine, and the Department of Obstetrics and Gynecology, University of Innsbruck, Austria.

Submitted June 21, 2001; accepted November 27, 2001.

Supported by the Austrian Fond zur Förderung der Wissenschaftlichen Forschung Grants No. S0716-MED (J.R.P.) and P-11693-MED (J.R.P.).

Address reprint requests to Josef R. Patsch, MD, Department of Medicine, University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria.

0026-0495/02/5105-0012$35.00/0

doi:10.1053/meta.2002.31991

reflects vascular function and which has been gaining acceptance as an early indicator of cardiovascular disease.[20]

MATERIALS AND METHODS

Patients

Study participants were recruited from a patient sample visiting the outpatient clinics at the Department of Gynecology, University of Innsbruck. Postmenopausal status was ascertained by measurement of serum estrogen (<0.02 ng/mL) and follicle-stimulating hormone (FSH) levels (>20 mU/mL). No patient had received hormone treatment for at least 6 months before entering the study. Patients with diabetes mellitus or liver, kidney, or thyroid disease were excluded; patients had no history of cardiovascular or cerebrovascular disease. Three of the patients had hypertension, one of whom was treated with doxazosin and metoprolol during the whole study. Potential adverse effects of metoprolol on HDL cholesterol, LDL cholesterol, and TG in plasma are compensated by the antagonistic effects of doxazosin.[21] Six patients had undergone (at least 1 year before entering the study) hysterectomy without oophorectomy. The study was approved by the local ethics committee. In accordance with institutional guidelines, all patients were aware of the investigational nature of the study and gave written consent.

Study Design and Statistics

All patients were examined immediately prior to and 3 months after implementation of HRT consisting of oral or transdermal estrogen (conjugated equine estrogen or estradiol) alone in patients after hysterectomy (n = 6), or estrogen and gestagen (medroxyprogesterone acetate or norethisteronacetate) (n = 7). Study parameters included basic clinical measurements, lipoprotein profile, postprandial lipemia, mass and activity of lipoprotein-metabolizing enzymes, body impedance analysis, and vascular endothelial function. Descriptive data are expressed as mean values ± SD. Consecutive measurements were compared by *t* test for paired samples using SPSS 6.1.1. (SPSS Inc, Chicago, IL) software.

Plasma Lipids, Lipoproteins, and Apolipoproteins

Plasma was collected after an overnight fast into vials containing EDTA to give a final concentration of 1 mg/mL. Cholesterol and TG were measured in plasma by enzymatic methods.[22,23] Quantification of HDL, HDL_2, and HDL_3 cholesterol included the same enzymatic methods in combination with a stepwise precipitation procedure to remove apoB-containing lipoproteins and HDL_2, respectively.[24,25] Plasma apoA-I and apoB were quantified by automated turbidimetric procedures (Roche Diagnostics, Mannheim, Germany). LDL size was analyzed on Lipoprotein Fractionation System-Lipogel (Zaxis, Hudson, OH) according to the user's manual. Relative retardation factor (relative Rf) was calculated by dividing Rf values of the lipoprotein-independent band running between LDL and HDL by the Rf value of the respective LDL band. Two control lanes were run on each gel containing plasma of well-defined patients displaying LDL pattern A and B, respectively.

Postprandial Lipemia

Immediately after donating postabsorptive blood, patients ingested a liquid fatty meal described in detail previously.[11] Briefly, the test meal contained per square meter of body surface area 65.0 g fat with a polyunsaturated to saturated fatty acids ratio of 0.06, 24 g carbohydrate, 4.75 g protein, and 240 mg cholesterol. TG were determined 0, 2, 4, 6, and 8 hours postprandially. The magnitude of postprandial lipemia was quantified by calculating the area under the postprandial TG curve normalized to the fasting level.[11]

Lipid Transfer Proteins (CETP and PLTP)

CETP mass was quantified using an enzyme-linked immunosorbent assay (ELISA).[26] CE transfer was measured as described by Kaser et al.[27] Briefly, CE transfer from exogenous HDL to apoB-containing lipoproteins was measured by incubating whole plasma with radiolabeled HDL (<7% of sample HDL cholesterol) and Na-iodoacetat as LCAT-inhibitor (Sigma, St Louis, MO) for 3 and 16 hours, reflecting initial rate and total transfer of CE, respectively. ApoB-containing lipoproteins were precipitated with dextranesulphate/$MgCl_2$. CE transfer was measured as the rate of total radiolabeled CE transfered to apoB-containing lipoproteins compared to controls stored at 4°C. Results are expressed as the percentage decrease of ^{3}H-CE in the supernatant of total radiolabeled CE. PLTP activity in plasma was measured by a previously described lipoprotein-independent radiometric assay.[28] Briefly, radiolabeled L-α-dipalmitoyl-phosphatidylcholine was incorporated in unilamellar liposomes, which were used as donor particles. Human HDL_3 isolated by zonal ultracentrifugation[29] were used as acceptor particles. At the end of the incubation period, liposomes were separated from HDL_3 by precipitation using heparin-$MnCl_2$ and subsequent centrifugation. The supernatant containing the HDL_3 particles was analyzed by liquid scintillation counting. Values are given as percent PLTP activity of a normolipidemic plasma pool (%NP).

Lipoprotein Lipase Activity

Patients were injected in the postabsorptive state intravenously with 2,280 U/m ^{2} heparin (Novo Industri A/S, Bagsvaerd, Denmark) to release lipoprotein lipase (LPL) into the circulation, and postheparin plasma was collected after 5 minutes. Measurement of LPL activity was performed as described.[30] Briefly, sonicated emulsions of radiolabeled trioleylglycerol (Amersham, Arlington Heights, IL) in phosphatitylcholine and gum arabic were employed as substrates for estimation of LPL activity. Heat-inactivated fasted rat serum was added as a source of apoC-II. Hepatic lipase (HL) was inhibited by goat anti-HL IgG. Activity is expressed in milliunits, which correspond to 1 nmol of fatty acids released per minute.

Body Impedance Analysis

Multiple-frequency bioelectrical impedance measurements using B.I.A. 2000 - M (Data Input, Frankfurt, Germany) were performed according to the user's manual. Briefly, resistance, reactance, and phase angle of injected currents of different frequencies were measured using 2 electrodes placed on the anterior surface of the right ankle and the dorsal surface of the right wrist, respectively. Total body water and lean body mass were determined using Nutri 4 software (Data Input) and used for calculation of body fat.

Flow-Mediated Dilation

FMD measurements were performed as described by others.[31] Briefly, the diameter of the brachial artery was measured in triplicate at rest, during reactive hyperemia (FMD), and after administration of 0.4 mg sublingual nitroglycerin (GTN), using a high-resolution ultrasound machine with a 10.0-MHz linear array transducer (VST-Masters, Diasonics, Santa Clara, CA). The percentage of the diameter of the endothelium-dependent (FMD) and of the endothelium-independent vasodilation (GTN) to the at-rest diameter, respectively, was calculated. Adjusted FMD (aFMD) and adjusted GTN (aGTN) were calculated by dividing percentage values of FMD- and GTN-induced vasodilation by the baseline vessel diameter.

RESULTS

Subjects

Most patients exhibited apoE genotype E3/3 (n = 8), 4 patients had apoE2/3, and 1 apoE3/4. None displayed apoE

Table 1. Subject Characteristics Before Treatment With HRT (N = 13)

	Mean ± SD (n=13)	Range
Age (yr)	52 ± 2	47-56
Height (cm)	164 ± 5	155-171
Body weight (kg)	73.3 ± 7.1	54-104
BMI (kg/m^2)	27.3 ± 5.5	20.5-36.0
Body fat (%)	32.3 ± 9.0	21.9-45.5
SBP (mm Hg)	127 ± 25	100-190
DPB (mm Hg)	80 ± 15	60-120

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

genotypes 2/2 or 4/4. Basic clinical characteristics of all women are shown in Table 1. HRT did not cause any significant change in body mass index or blood pressure.

Lipoproteins

Other than a tendency for HDL_2 cholesterol to be higher, the only significant changes in plasma lipid and apolipoprotein concentrations were a 17% increase in fasting TG and a 16 % increase in apoA-I. Levels of total, LDL, and HDL cholesterol were not influenced by HRT, respectively (Table 2), but LDL size decreased in patients treated with estrogen alone (group A: 1.161 ± 0.020 *v* 1.154 ± 0.024 relative Rf, $P < .05$).

Lipid Transfer Proteins

Although there was no change in CETP mass or in initial rate of CE transfer before and after HRT, total transfer of CE from exogenous HDL to apoB-containing lipoproteins increased significantly by 10% (Table 3 and Fig 1). PLTP activity was not changed by HRT considering all patients together, but tended to be increased in patients treated with estrogen alone (110.8% ± 5.0% NP before HRT *v* 116.8% ± 8.8% NP after HRT, $P = .070$) (Table 3 and Fig 2).

Postprandial Lipemia and LPL

Although no changes in plasma LPL activity were observed, the magnitude of postprandial lipemia tended to increase in all patients (from 1,483 ± 753 mg/dL/8 h to 1,660 ± 815 mg/dL/8 h, $P = .129$) and reached significance in women treated with estrogen alone (from 1,475 ± 930 mg/dL/8 h to 1,737 ± 756

Table 2. Plasma Lipoprotein and Apolipoprotein Parameters Before and 3 Months After HRT in All Patients (N = 13)

Parameter	Before HRT (mean ± SD)	After HRT (mean ± SD)	*P* Value*
Total cholesterol (mg/dL)	197 ± 38	201 ± 25	
LDL cholesterol (mg/dL)	109 ± 32	108 ± 23	
HDL cholesterol (mg/dL)	66 ± 18	67 ± 16	
HDL_2 cholesterol (mg/dL)	9 ± 15	12 ± 7	
HDL_3 cholesterol (mg/dL)	56 ± 14	54 ± 12	
TG (mg/dL)	115 ± 39	134 ± 40	.037
apoA-I (mg/dL)	147 ± 22	171 ± 30	.024
apoB (mg/dL)	94 ± 23	103 ± 15	
Lipoprotein (a) (mg/dL)	39 ± 67	33 ± 54	

*Only *P* values ≤ .05 are presented.

Table 3. Plasma CETP Concentration, CE Transfer, LPL, and PLTP Activity Before and 3 Months After HRT in All Patients (N = 13)

Parameter	Before HRT (mean ± SD)	After HRT (mean ± SD)	*P* Value*
CETP mass (μg/mL)	1.14 ± 0.22	1.21 ± 0.31	
Initial CE transfer (%)	21.6 ± 11.0	22.2 ± 10.7	
Total CE transfer (%)	50.8 ± 13.7	56.0 ± 11.5	.025
LPL activity (mU/mL)	258 ± 100	240 ± 77	
PLTP activity (%NP)	110.4 ± 14.7	111.7 ± 14.5	

*Only *P* values ≤ .05 are presented.

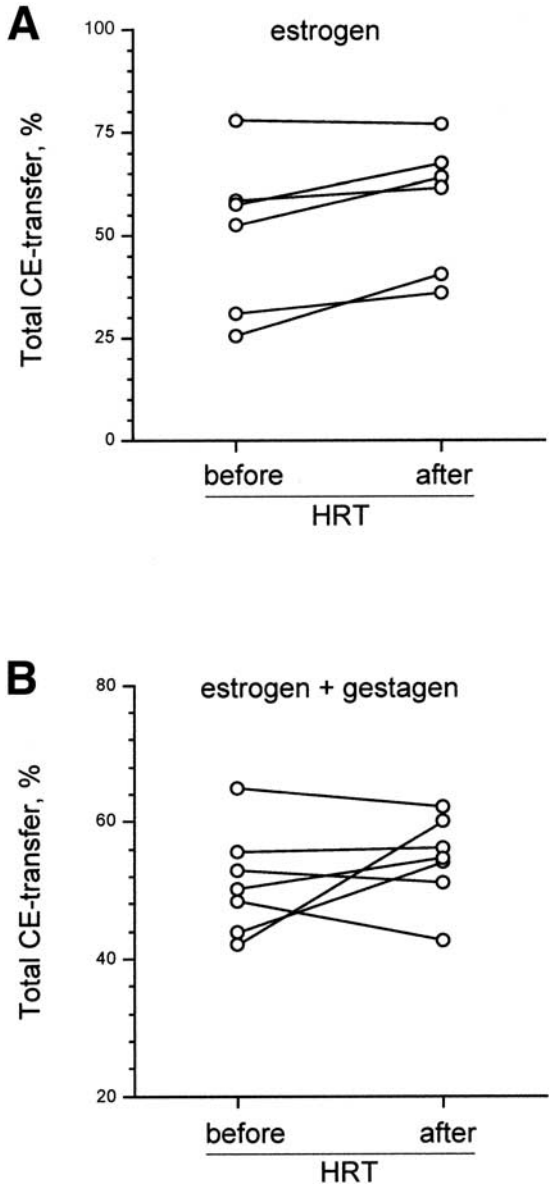

Fig 1. Total CE transfer in patients before and 3 months after treatment with estrogen (A) or estrogen opposed by gestagen (B). Each line represents measurements within a single patient. Results are expressed as the % decrease of ^{3}H-CE in the supernatant of total radiolabeled CE.

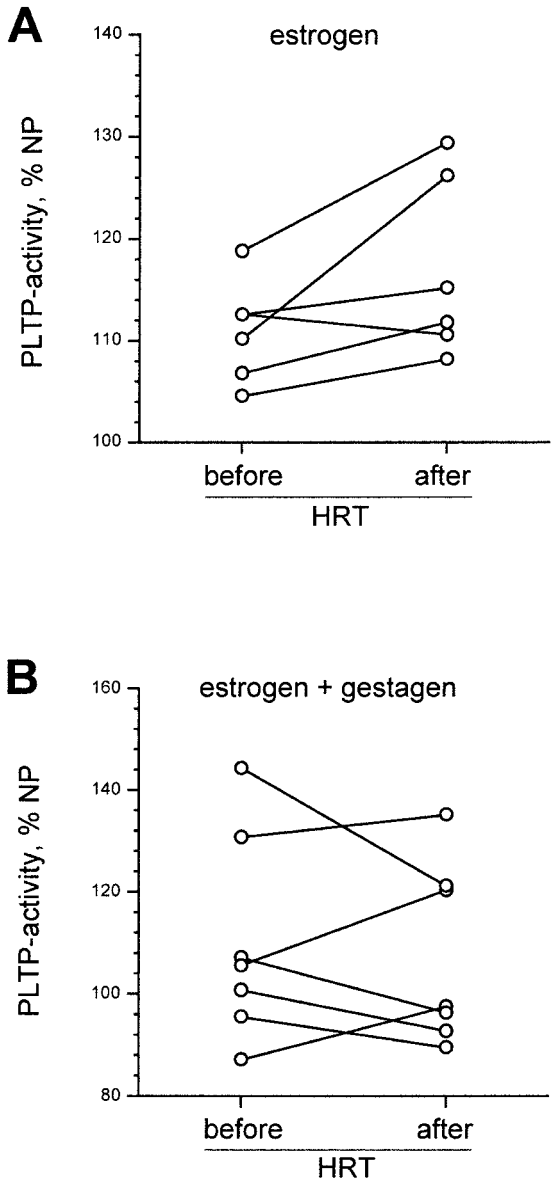

Fig 2. PLTP activity in patients before and 3 months after treatment with estrogen (A) or estrogen opposed by gestagen (B). Each line represents measurements within a single patient. Values are given as % PLTP activity of a normolipidemic plasma pool (%NP).

mg/dL/8 h, n = 6, $P = .025$) but not in patients treated with estrogen and gestagen (from 1,402 ± 615 mg/dL/8 h to 1,584 ± 936 mg/dL/8 h, n = 7, $P = .531$).

Vascular Functions

None of the parameters measured, including FMD- and GTN-induced vasodilation with and without adjustment for baseline vessel diameter, were influenced by HRT (Table 4).

DISCUSSION

All lipoprotein-directed effects of HRT in postmenopausal women were more pronounced in women taking estrogen alone (group A) when compared to patients treated with a combination of estrogen and gestagen (group B). Group A patients differed from patients of group B by having a history of hysterectomy before entering the study. However, hysterectomy per se does not have an influence on lipid and lipoprotein metabolism.[32] Thus, it may be the additional use of gestagen which, at least partially, sets off the effects produced by estrogen therapy.

In our study the magnitude of postprandial lipemia increased in patients treated with estrogen. This finding may be partially at variance with those of some previous studies, where no changes in postprandial TG levels or even a better clearance of remnant particles was observed. However, the degree of adiposity in our study patients was somewhat variable, which might account for some of these discrepancies. We hypothesize that the increased magnitude of postprandial lipemia reported in this study is due to the well-known estrogen-induced increase in VLDL secretion,[33] which adds to the magnitude of overall lipemia during the postprandial phase. The preferential clearance of chylomicron TG by LPL was shown to lead to accumulation of hepatogenous VLDL in the postprandial period.[2,34] This mechanism most likely becomes increasingly important with the well-documented increase in VLDL levels due to HRT.

Surprisingly, no effects on FMD were observed, although several studies have reported beneficial effects of HRT on vascular function in postmenopausal women.[35,36] This discrepancy may be explained by several of the exclusion criteria we applied. Most of the previous studies were based on patients with highly elevated plasma cholesterol or TG levels, or on patients with history of cardiovascular or cerebrovascular disease. This study is based on a homogeneous group of patients without grossly elevated levels of plasma lipids including total cholesterol, LDL cholesterol, and TG. Exclusion criteria included those mentioned above, but also other diseases potentially affecting lipoprotein metabolism.

One of the major changes seen in this study with HRT was an increase of plasma apoA-I concentration. Together with a tendency of PLTP to increase, the early steps in reverse cholesterol transport appear to be augmented with HRT. In patients treated with estrogen alone we found an increase in HDL cholesterol of 3 mg/dL, quite similar to the increase of 5.6 mg/dL in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial of 175 patients.[3] However, this change did not reach the level of significance, possibly due to the small num-

Table 4. Flow-Mediated Dilation Before and 3 Months After HRT in All Patients (N = 13)

	Before HRT (mean ± SD)	After HRT (mean ± SD)
FMD (%)	2.90 ± 3.33	3.21 ± 3.98
GTN (%)	13.68 ± 4.30	13.50 ± 4.88
aFMD (%/cm)	7.27 ± 8.77	8.18 ± 3.98
aGTN (%/cm)	35.26 ± 12.56	33.80 ± 14.24
Vessel diameter (cm)	0.39 ± 0.05	0.41 ± 0.05

Abbreviations: FMD, flow-mediated dilation; GTN, nitroglycerin-induced dilation; aFMD, adjusted flow-mediated dilation; aGTN, adjusted nitroglycerin-induced dilation.

ber of patients in our study and/or by the employment of different HRT regimens. There may be quantitative differences between oral and transdermal administration of HRT in postmenopausal women with attenuated effects in patients treated with transdermal estrogens. However, it has been shown in several studies that both regimens have the same qualitative effects on lipid metabolism.[37,38] Additionally, subtle differences are found concerning androgenicity between medroxyprogesterone and norethisterone. However, no significant modifications in lipid and lipoprotein metabolism could be observed in a corresponding study by Perrone et al.[39]

The expected rise in HDL cholesterol may be attenuated in our clinical setting due to the observed increase in CE transfer from HDL to apoB-containing lipoproteins. Plasma newly synthesized cholesteryl ester transfer (NCET) was not enhanced by HRT in postmenopausal women.[18] NCET was shown to be closely related with net CE mass transfer from HDL to apoB-containing lipoproteins.[19] However, a possible change in CE transfer may be covered by changes in LCAT activity in this system. The influence of LCAT activity was excluded in the CE-transfer assay used in this study employing Na-iodoacetat as an LCAT-inhibitor.

The observed enhancement of CE transfer in our study was not brought about by an increase in CETP concentration, which is in agreement with previous studies where HRT did not affect CETP concentration. From these studies it was concluded that CETP was not involved in the HRT-induced effects on HDL cholesterol.[17,40] However, in our study we additionally measured CE transfer from HDL to apoB-containing lipoproteins. The observed increased CE transfer may be brought about by an increased substrate availability, ie, increased fasting and postprandial TG. Unpublished experiments from our laboratory indicate that the initial rate of CE transfer is influenced mainly by CETP concentration, whereas total rate of CE transfer is primarily determined by the presence of TG-rich acceptor lipoproteins.

The hypertriglyceridemia induced by estrogen is due to increased synthesis of large TG-rich VLDL particles[41] as opposed to hypertriglyceridemia caused by impaired VLDL catabolism.[42] Moreover, estrogen increases the fractional catabolic rate, leading to a lower plasma residence time and thereby reducing the length of time for potential oxidation and remodeling.[43] Our study showed enhanced CE transfer from HDL to TG-rich lipoproteins with HRT, internally consistent with our finding of a shift of the LDL pattern from large towards more dense LDL.[44,45] The enhanced CE transfer as well as the shift in LDL particle size, although considered potentially atherogenic,[46] may not exert atherogenic effects in the setting of HRT described here, as the plasma residence time of LDL is drastically reduced.

In summary, HRT as described in this study showed subtle but discrete changes in lipoprotein patterns but no direct effects on vascular function reflected by measurement of FMD. HRT in postmenopausal women leads to a pronounced increase in apoA-I without an equivalent rise in HDL cholesterol. This might be explained by the enhancement of CE transfer from HDL to apoB-containing lipoproteins, which has both proatherogenic aspects like the generation of small dense LDL, as well as antiatherogenic aspects, like the stimulation of reverse cholesterol transport. We therefore feel that our study helps to explain how HRT may protect women against atherosclerosis.

REFERENCES

1. Grodstein F, Stampfer MJ, Manson JE, et al: Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. N Engl J Med 335:453-461, 1996

2. Walsh BW, Schiff I, Rosner B, et al: Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 325:1196-1204, 1991

3. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial. JAMA 273:199-208, 1995

4. Hulley S, Grady D, Bush T, et al: Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. JAMA 280:605-613, 1998

5. Patsch JR, Miesenböck G, Hopferwieser T, et al: Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 12:1336-1345, 1992

6. Weintraub MS, Grosskopf I, Rassin T, et al: Clearance of chylomicron remnants in normolipidaemic patients with coronary artery disease: Case control study over three years. Br Med J 312:936-939, 1996

7. Julius U, Fritsch H, Fritsch W, et al: Impact of hormone replacement therapy on postprandial lipoproteins and lipoprotein(a) in normolipidemic postmenopausal women. Clin Invest 72:502-507, 1994

8. Westerveld HT, Kock LA, van Rijn HJ, et al: 17β-Estradiol improves postprandial lipid metabolism in postmenopausal women. J Clin Endocrinol Metab 80:249-253, 1995

9. Weintraub M, Grosskopf I, Charach G, et al: Hormone replacement therapy enhances postprandial lipid metabolism in postmenopausal women. Metabolism 48:1193-1196, 1999

10. Knopp RH, Zhu X, Bonet B: Effects of estrogens on lipoprotein metabolism and cardiovascular disease in women. Atherosclerosis 110: S83-91, 1994 (suppl)

11. Patsch JR, Karlin JB, Scott LW, et al: Inverse relationship between blood levels of high density lipoprotein subfraction 2 and the magnitude of postprandial lipemia. Proc Natl Acad Sci USA 80:1449-1453, 1983

12. Mann CJ, Yen FT, Grant AM, et al: Mechanisms of plasma cholesteryl ester transfer in hypertriglyceridemia. J Clin Invest 88: 2059-2066, 1991

13. Riemens S, van Tol A, Sluiter W, et al: Elevated plasma cholesteryl ester transfer in NIDDM: Relationships with apolipoprotein B-containing lipoproteins and phospholipid transfer protein. Atherosclerosis 140:71-79, 1998

14. Föger B, Ritsch A, Doblinger A, et al: Relationship of plasma cholesteryl ester transfer protein to HDL cholesterol. Studies in normotriglyceridemia and moderate hypertriglyceridemia. Arterioscler Thromb Vasc Biol 16:1430-1436, 1996

15. Ritsch A, Drexel H, Amann FW, et al: Deficiency of cholesteryl ester transfer protein due to a novel molecular defect: Dissociation of transport of cholesteryl esters and triglycerides in plasma. Arterioscler Thromb Vasc Biol 17:3433-3441, 1997

16. Koizumi J, Inazu A, Yagi K, et al: Serum lipoprotein lipid concentration and composition in homozygous and heterozygous patients with cholesteryl ester transfer protein deficiency. Atherosclerosis 90:189-196, 1991

17. Sacks FM, McPherson R, Walsh BW: Effect of postmenopausal estrogen replacement on plasma Lp(a) lipoprotein concentrations. Arch Intern Med 154:1106-1110, 1994

18. Lewis-Barned NJ, Sutherland WH, Walker RJ, et al: Plasma cholesterol esterification and transfer, the menopause, and hormone replacement therapy in women. J Clin Endocrinol Metab 84:3534-3538, 1999

19. Sutherland WH, Walker RJ, Lewis-Barned NJ, et al: Plasma cholesteryl ester transfer in patients with non-insulin dependent diabetes mellitus. Clin Chim Acta 231:29-38, 1994

20. Celermajer DS: Testing endothelial function using ultrasound. J Cardiovasc Pharmacol 32:S29-32, 1998 (suppl)

21. Suter PM, Vetter W: Metabolic effects of antihypertensive drugs. J Hypertens 13:S11-17, 1995 (suppl)

22. Nagele U, Hagele EO, Sauer G, et al: Reagent for the enzymatic determination of serum total triglycerides with improved lipolytic efficiency. J Clin Chem Clin Biochem 22:165-174, 1984

23. Siedel J, Hagele EO, Ziegenhorn J, et al: Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem 29:1075-1080, 1983

24. Warnick GR, Benderson J, Albers JJ: Quantitation of high density lipoprotein subclasses after seperation by dextran sulfate and Mg^{2+} precipitation. Clin Chem 28:1574, 1982 (abstr)

25. Patsch W, Brown SA, Morrisett JD, et al: A dual-precipitation method evaluated for measurement of cholesterol in high-density lipoprotein subfractions HDL_2 and HDL_3 in human plasma. Clin Chem 35:265-270, 1989

26. Ritsch A, Doppler W, Pfeifhofer C, et al: Cholesteryl ester transfer protein gene expression is not specifically regulated by CCAAT/enhancer-binding protein in HepG2-cells. Atherosclerosis 146:11-18, 1999

27. Kaser S, Ebenbichler CF, Wolf HJ, et al: Lipoprotein profile and cholesteryl ester transfer protein in neonates. Metabolism 50:723-728, 2001

28. Föger B, Santamarina-Fojo S, Shamburek RD, et al: Plasma phospholipid transfer protein. Adenovirus-mediated overexpression in mice leads to decreased plasma high density lipoprotein (HDL) and enhanced hepatic uptake of phospholipids and cholesteryl esters from HDL. J Biol Chem 272:27393-27400, 1997

29. Patsch JR, Patsch W: Zonal ultracentrifugation. Methods Enzymol 129:3-26, 1986

30. Miesenböck G, Holzl B, Foger B, et al: Heterozygous lipoprotein lipase deficiency due to a missense mutation as the cause of impaired triglyceride tolerance with multiple lipoprotein abnormalities. J Clin Invest 91:448-455, 1993

31. Celermajer DS, Sorensen KE, Gooch VM, et al: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 340:1111-1115, 1992

32. Kritz-Silverstein D, Barrett-Connor E, Wingard DL: Hysterectomy, oophorectomy, and heart disease risk factors in older women. Am J Public Health 87:676-680, 1997

33. Walsh BW, Sacks FM: Effects of low dose oral contraceptives on very low density and low density lipoprotein metabolism. J Clin Invest 91:2126-2132, 1993

34. Schneeman BO, Kotite L, Todd KM, et al: Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. Proc Natl Acad Sci USA 90:2069-2073, 1993

35. Lieberman EH, Gerhard MD, Uehata A, et al: Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Intern Med 121:936-941, 1994

36. McCrohon JA, Adams MR, McCredie RJ, et al: Hormone replacement therapy is associated with improved arterial physiology in healthy post-menopausal women. Clin Endocrinol Oxf 45:435-441, 1996

37. Rozenberg S, Ylikorkala O, Arrenbrecht S: Comparison of continuous and sequential transdermal progestogen with sequential oral progestogen in postmenopausal women using continuous transdermal estrogen: Vasomotor symptoms, bleeding patterns, and serum lipids. Int J Fertil Womens Med 42:376-387, 1997

38. Chen FP, Lee N, Soong YK, et al: Comparison of transdermal and oral estrogen-progestin replacement therapy: Effects on cardiovascular risk factors. Menopause 8:347-352, 2001

39. Perrone G, Falaschi P, Capri O, et al: Hormonal and metabolic effects of transdermal estradiol/progestagen administration in postmenopausal women. Int J Fertil Menopausal Stud 39:202-207, 1994

40. Tilly-Kiesi M, Kahri J, Pyorala T, et al: Responses of HDL subclasses, Lp(A-I) and Lp(A-I:A-II) levels and lipolytic enzyme activities to continuous oral estrogen-progestin and transdermal estrogen with cyclic progestin regimens in postmenopausal women. Atherosclerosis 129:249-259, 1997

41. Campos H, Walsh BW, Judge H, et al: Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. J Clin Endocrinol Metab 82: 3955-3963, 1997

42. Packard CJ, Munro A, Lorimer AR, et al: Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. J Clin Invest 74:2178-2192, 1984

43. Knopp RH, Zhu X: Multiple beneficial effects of estrogen on lipoprotein metabolism. J Clin Endocrinol Metab 82:3952-3954, 1997

44. van der Mooren MJ, de Graaf J, Demacker PN, et al: Changes in the low-density lipoprotein profile during 17β-estradiol-dydrogesterone therapy in postmenopausal women. Metabolism 43:799-802, 1994

45. Campos H, Sacks FM, Walsh BW, et al: Differential effects of estrogen on low-density lipoprotein subclasses in healthy postmenopausal women. Metabolism 42:1153-1158, 1993

46. Stampfer MJ, Krauss RM, Ma J, et al: A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 276:882-888, 1996