Effects of Continuous Low-Dosage Hormonal Replacement Therapy on Lipoprotein Metabolism in Postmenopausal Women

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The effects on lipoprotein metabolism of female hormone replacement therapy (HRT) for 7 weeks with combined low dosages of a widely used oral progestin and estrogen combination, medroxyprogesterone acetate ([MPA] 2.5 mg/d) and conjugated equine estrogen ([CEE] 0.625 mg/d), were studied in six postmenopausal women. To investigate the mechanism of the reduction of low-density lipoprotein (LDL) cholesterol by HRT, the kinetics of very–low-density lipoprotein (VLDL) and LDL apolipoprotein (apo) B turnover were studied by injection of autologous ^{131}I-labeled VLDL and ^{125}I-labeled LDL under control conditions and again in the fourth week of HRT. HRT induced (1) a 12% ± 4% ($P < .02$) reduction of the cholesterol content of LDL of S_f 0-12, which was attributable to a 15% ± 5% decrease in the mean ratio of cholesterol to apo B (1.3 ± 0.1 *v* 1.5 ± 0.1, $P < .025$), and (2) a 13% ± 4% increase in the mean fractional catabolic rate (FCR) of LDL apo B (0.34 ± 0.03 *v* 0.30 ± 0.02 pools/d, respectively, $P \pm .05$). However, there were no significant changes in mean values for (1) pool size (42 ± 4 *v* 43 ± 3 mg/kg) or production rate (14 ± 0.5 *v* 13 ± 0.9 mg/kg/d, $P > .1$) of LDL apo B or (2) pool size (2.5 ± 0.6 *v* 2.8 ± 0.6 mg/kg), FCR (8.0 ± 2.0 *v* 8.1 ± 1.7 pools/d) or production rate (16 ± 4 *v* 19 ± 2 mg/kg/d, $P > .04$) of VLDL apo B. HRT increased high-density lipoprotein (HDL) cholesterol concentration significantly (by 16% to 18%, $P < .05$), whereas the mean ratio of plasma total cholesterol to HDL cholesterol decreased by 19% ± 3% ($P < .005$). HRT favorably influenced the overall plasma lipoprotein lipid vascular risk profile while significantly altering both the composition and fractional catabolism of LDL.

APPROXIMATELY 36 million American women currently lack estrogen and progesterone as a result of menopause.[1,2] Their numbers are expected to increase rapidly, so that by the year 2006 approximately 70 million Americans and 700 million women world wide will be of menopausal age.[1-3] Coronary heart disease (CHD), the major mortality risk of postmenopausal women, has a mortality rate that markedly exceeds that of either breast cancer or hip fracture by an order of magnitude.[4] Elevated plasma cholesterol[5,6] tends to accompany the postmenopausal increase in CHD[7,8] and contributes to the loss of relative immunity from CHD enjoyed by premenopausal women as compared with men of similar age.[9] Estrogen (± progestin) replacement appears to reduce all-cause mortality by 11% to 20% and CHD mortality by 50%.[10-12] However, prolonged estrogen therapy that is unopposed by natural or synthetic progestin increases the risk of uterine neoplasia by 1.7-fold to eightfold.[13,14] Although intermittent administration of progestin together with estrogen reduces the relative risk of endometrial cancer to ≤ 1.0,[14,15] such therapy tends to result in poor patient compliance[16] because of bothersome and often painful scheduled vaginal withdrawal bleeding,[13,14,17] premenstrual-like symptoms, and anxiety about uterine cancer and/or return of fertility.[16,18,19] However, continuous daily administration of hormone replacement therapy (HRT) induces endometrial atrophy and largely eliminates vaginal bleeding (usually immediately or over time), which facilitates compliance and long-term prophylaxis against CHD and osteoporosis.[20-25] Estrogen/progestin replacement therapy appears to decrease the risk of CHD by various mechanisms, including reductions in plasma low-density lipoprotein (LDL) cholesterol[26] and increases in high-density lipoprotein (HDL) cholesterol,[27,28] as well as other mechanisms.[29-31] Although the combination of continuous low dosages of medroxyprogesterone acetate (MPA) and conjugated equine estrogen (CEE) has recently been recommended for HRT in menopause,[32] the mechanism whereby the combination influences the metabolism of major apolipoprotein (apo) B–containing lipoproteins and in particular decreases LDL cholesterol[27] has not been investigated. We have therefore studied the effects of continuous oral low-dosage MPA + CEE on the kinetics of apo B metabolism of plasma LDL and very–low-density lipoprotein (VLDL) in estrogen-deficient postmenopausal women.

SUBJECTS AND METHODS

Subjects

Six participants who had experienced typical menopausal symptoms, were amenorrheic for at least 6 months, and had serum follicle-stimulating hormone levels greater than 70 U/L before entering the study were recruited from University Hospital outpatient clinics (Table 1). Baseline fasting plasma cholesterol concentrations during phase I American Heart Association diets before entry into the study were ≥200 mg/dL, the cutoff point of eligibility for dietary treatment recommended by the National Cholesterol Education Program.[33] One subject was treated with L-thyroxine 0.15 mg/d for primary hypothyroidism. Two subjects were treated throughout the entire study with antihypertensive medication, one with triamterene 50 mg and hydrochlorothiazide 25 mg daily and the other with hydrochlorothiazide 50 mg and amiloride 5 mg daily. Subjects received no other medications known to affect lipid metabolism, and intake of ethanol was less than 30 mL/d for the month preceding each turnover study. All subjects had normal fasting concentrations of serum glucose, glutamic oxaloacetic transaminase, thyroxine, creatinine, calcium, albumin, sodium, potassium, chloride, and bicarbonate. Hematologic and urine analyses were normal. The experimental protocol

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Table 1. Characteristics of Postmenopausal Subjects

Subject No.	Age (yr)	Height (cm)	Weight (kg)	Cholesterol (mg/dL)		Triglycerides (mg/dL)		Lp(a) (mg/dL)	
				Control	Treated	Control	Treated	Control	Treated
1	38	163	65	255	248	88	95	12	13
2	59	176	79	182	178	138	146	3.4	2.5
3	65	155	60	236	213	181	198	23	20
4	61	161	79	263	232	292	224	6.2	5.6
5	71	160	52	232	244	120	120	16	16
6	71	156	67	221	205	112	120	33	27
Mean ± SE	61 ± 5	162 ± 3	67 ± 4	232 ± 12	220 ± 11	155 ± 30	151 ± 21	16 ± 5	14 ± 4

NOTE. Values for each subject are the mean of four fasting blood plasma samples obtained over 3 weeks during a low-fat, low-cholesterol diet for each control and treatment period.

approved by The Standing Committee on Human Research of The University of Western Ontario was explained to each subject, and informed consent was obtained in writing.

Subjects were maintained on moderately low-fat, low-cholesterol diets[34] that contained approximately 27% of energy from fat (mean ratio of polyunsaturated:monounsaturated:saturated fat, ~0.7:1.0:1.0; 150 mg cholesterol/d), 21% from protein, and 52% from carbohydrate. During hospitalization for the first day only of each turnover study, the diet was unchanged except that the carbohydrate content was increased at the expense of fat to maintain caloric intake. Fat intake was reduced to less than 5 g/d to eliminate the contribution of intestinal lipoproteins to plasma triglyceride-rich lipoproteins.[35]

Samples of venous blood for determination of plasma total and HDL cholesterol, total triglyceride, and lipoprotein(a) [Lp(a)] concentrations were obtained from a forearm vein (after a 12-hour fast) from each subject on four occasions at approximately weekly intervals (1) during the initial 3-week control period before initiation of HRT and (2) during HRT (weeks 3 to 6 of the 7-week course of MPA 2.5 mg/d + CEE 0.625 mg/d). Subjects underwent studies of the kinetics of VLDL and LDL apo B turnover during the control period and again during the last 16 days of treatment with MPA + CEE. All subjects received potassium iodide 300 mg/d for 3 days before and 13 days after reinjection of the radiolabeled lipoproteins.

Preparation of Labeled Lipoproteins and Kinetic Studies

Fasting blood plasma (1.5 mg EDTA/mL) for isolation of VLDL and LDL was obtained from each subject 6 days before each turnover study. Procedures used for preparation of labeled lipoproteins have been previously described.[36-40] VLDL (S_f 60-400) and LDL (S_f 0-12) were radiolabeled with ^{131}I (Merck-Frosst Canada, Point Claire-Dorval, Quebec) and ^{125}I (Amersham, Oakville, Ontario, Canada), respectively. Ninety-two percent of the radioisotope ^{131}I was bound to VLDL protein, 2% was associated with lipids, and 5% remained unbound; VLDL apo B accounted for 37% of ^{131}I bound to VLDL protein. Ninety-eight percent of the radioisotope ^{125}I was bound to LDL protein, 1% was associated with lipids, and 1% remained unbound; LDL apo B accounted for 92% of ^{125}I bound to LDL protein.

Experimental Protocol

Each recipient was fasted 12 hours before receiving serial bolus injections of ^{131}I-labeled VLDL 30 μCi and ^{125}I-labeled LDL 100 μCi in 2 to 3 mL normal saline via a hand or forearm vein. Venous blood samples (each 25 mL, 1.5 mg EDTA/mL) were obtained over 16 days after injection at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 120, 216, 288, and 384 hours and placed on ice before immediate separation of plasma. Plasma VLDL (S_f 60-400), intermediate-density lipoprotein ([IDL] S_f 12-60), and LDL (S_f 0-12) were fractionated,[37,41] apo B was isolated from each lipoprotein fraction by isopropanol precipitation, and specific activity was determined as previously described.[42]

Analyses

The structure of the kinetic model and equations used to calculate kinetic parameters have been described previously.[38] The principal assumptions of this model are that (1) apo B is an integral part of VLDL, IDL, and LDL, is nonexchangeable, and therefore provides an accurate measure of metabolism of these lipoproteins; (2) metabolism of the tracer is identical to that of the tracee; (3) production rate or flux of VLDL apo B represents input of apo B primarily into the larger, rapid-turnover, primary metabolic compartment (pool 1); (4) all apo B catabolism occurs from pool 1 either directly or after passage through pool 2; (5) all subpopulations of VLDL are isolated for labeling, and the initial injected activity is proportional to the mass of apo B in each population; (6) the two-pool model for LDL apo B is characterized by a plasma compartment and extravascular exchange compartment; and (7) at the time of injection all VLDL and LDL apo B radioactivity is introduced into the plasma compartment, and irreversible loss of LDL apo B occurs from the same compartment.

Specific-activity curves for apo B of reinjected VLDL and LDL were analyzed and kinetic parameters calculated as described in earlier studies in humans.[37-38,43-45] This yielded values for flux rates through pool 1, irreversible fractional catabolic rate ([FCR] = k_A, assuming that catabolism from other pools is negligible),[43,44] and mass in pool 1. The intravascular content of VLDL apo B was also calculated independently from the plasma concentration of VLDL apo B multiplied by the plasma volume taken as 45 mL/kg body weight.[46] The amount of LDL apo B derived from IDL apo B was calculated as the area under the ^{131}I-LDL apo B specific-activity curve divided by the area under the ^{131}I-IDL apo B specific-activity curve.[39,47]

Plasma VLDL and IDL apo B concentrations were measured immunoturbidimetrically using a Tina-quant apo B kit obtained from Boehringer Mannheim Canada (Laval, Quebec). The assay was standardized to LDL isolated by ultracentrifugation (d 1.040 to 1.063) in which it was determined that apo B was the only protein present. Protein content was determined by the modified Lowry procedure.[48] Lp(a) level was measured using a Macra Lp(a) Kit obtained from Terumo Medical (Elkton, MD). Intraassay coefficients of variation for apo B and Lp(a) were 1.8% and 4.7%, respectively. Concentrations of cholesterol and triglycerides in the chloroform-methanol extract of whole plasma were determined as previously described,[38] except that diagnostic kits from Boehringer Mannheim Diagnostica, Montreal, Quebec (C-system Kit for cholesterol and Test Combination Kit for triglycerides), were used

Table 2. Effects of HRT on Cholesterol Levels (mg/dL) of Plasma Lipoproteins in Fasted Subjects During Turnover Studies

Subject No.	Whole Plasma		VLDL		IDL		LDL		HDL	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	252	252	1.0	3.0	18	21	179	162	54	66
2	174	178	8.9	8.9	17	22	113	104	35	43
3	236	201	17	15	24	27	160	113	35	46
4	263	217	30	13	42	28	152	141	39	35
5	247	248	6.0	2.1	18	13	149	140	74	93
6	209	197	3.0	2.5	12	15	155	134	39	43
Mean ± SE	230 ± 14	216 ± 12	11 ± 4	7.4 ± 2	23 ± 4	21 ± 2	151 ± 9	132 ± 9*	46 ± 6	54 ± 9†

NOTE. Values for each subject were based on fasting blood plasma samples obtained immediately before injection of autologous labeled ^{131}I-VLDL and ^{125}I-LDL at the beginning of each turnover study.

*Different from control, $P < .025$.

†Different from control, $P < .05$.

to measure cholesterol content of fasting plasma VLDL, IDL, and LDL and triglyceride content of VLDL during the turnover studies because of the small amounts of material available for assay. HDL cholesterol level was measured following precipitation with heparin-manganese chloride.[49]

Values from control and treatment periods were compared using Student's paired two-tailed t test.[50] Variance was expressed as standard error of the mean.

RESULTS

Concentrations of Lipoprotein Lipids and Apo B

The mean plasma concentration of cholesterol transported in plasma LDL (S_f 0-12) decreased significantly by 12% ± 4% during HRT as compared with the control value ($P < .025$; Table 2). However, the mean concentration of plasma total cholesterol decreased by only 5% to 6% because of the significant 16% ± 6% (54 ± 9 *v* 46 ± 6 mg/dL, $P < .05$) increase in HDL cholesterol. There was a significant 19% ± 3% decrease in the mean ratio of total cholesterol to HDL cholesterol (4.2 ± 0.5 *v* 5.1 ± 0.5, $P < .005$, HRT *v* control) and a 26% ± 5% decrease in the ratio of conventional LDL (S_f 0-20) cholesterol to HDL cholesterol (2.9 ± 0.4 *v* 3.8 ± 0.4, $P < .005$) during weeks 3 to 6 of HRT versus control conditions. The 10% ± 2% reduction in the mean cholesterol concentration of S_f 0-20 lipoproteins during HRT versus control (149 ± 11 *v* 166 ± 11 mg/dL, $P < .02$) was similar to the 12% ± 4% reduction observed for S_f 0-12 (Table 2). The mean concentration of Lp(a)[51,52] decreased by 9% ± 5% during HRT versus control (14 ± 4 *v* 16 ± 5 mg/dL, $.1 > P > .2$). The mean ratio of cholesterol to apo B of plasma LDL (S_f 0-12) was significantly lower for HRT than for control by 15% ± 5% (1.3 ± 0.1 *v* 1.5 ± 0.1, $P < .025$), whereas the ratio of triglyceride to apo B was 18% ± 9% higher (0.31 ± 0.03 *v* 0.27 ± 0.03, $.05 < P < .10$). Mean values for the ratio of cholesterol to apo B in plasma VLDL or IDL were not significantly different during HRT versus control (2.4 ± 0.6 *v* 3.3 ± 1.2 and 1.4 ± 0.1 *v* 1.4 ± 0.1, respectively, $P > .2$), nor were those for the ratio of triglycerides to apo B in VLDL and IDL (29 ± 8 *v* 25 ± 5 and 2.5 ± 0.2 *v* 2.5 ± 0.4, respectively, $P > .4$). Mean values for the concentration of apo B in plasma VLDL, IDL, and LDL were similar during HRT versus control (2.2 ± 0.4 *v* 2.2 ± 0.3, 12 ± 1 *v* 12 ± 1, and 81 ± 5 *v* 76 ± 5 mg/dL, respectively; Table 3). The intravascular content of the VLDL apo B pool calculated from the plasma concentration of VLDL apo B multiplied by estimated plasma volume yielded values that were less than those obtained for the mass of VLDL apo B in pool A, in agreement with results reported by Reardon et al.[37]

The mean plasma concentration of triglycerides transported in lipoproteins of S_f 0-12 during turnover studies was significantly greater during HRT versus control (30 ± 2 *v* 22 ± 3 mg/dL, $P < .05$). However, mean values for plasma total triglycerides (143 ± 20 *v* 155 ± 37 mg/dL), VLDL triglycerides (64 ± 16 *v* 83 ± 28 mg/dL), and triglycerides transported in lipoproteins of S_f 12-60 (IDL, 34 ± 4 *v* 32 ± 8 mg/dL) were not different during HRT versus control ($P > .5$).

Consistent with a steady state, there were no systematic changes during turnover studies in plasma concentrations of the most readily quantified indices, namely total protein content of LDL (mean coefficient of variation of 8.1%, corresponding to a mean SD of 7.0 mg/dL, n = 6 paired studies) and total protein content of VLDL (mean coefficient of variation of 16%, corresponding to a mean SD of 1.3 mg/dL, n = 6 paired studies).

Metabolism of Lipoproteins

Values for specific activities of ^{131}I-labeled apo B in VLDL, IDL, and LDL over 72 hours after injection of

Table 3. Effects of HRT on Apo B Levels (mg/dL) of Plasma Lipoproteins in Fasted Subjects During Turnover Studies

Subject No.	VLDL*		IDL*		LDL†	
	Control	Treated	Control	Treated	Control	Treated
1	1.0	1.3	17	11	85	93
2	2.8	2.9	10	11	56	72
3	3.4	3.7	12	14	78	94
4	2.1	2.0	15	14	91	87
5	1.9	1.5	11	8.3	66	63
6	1.9	1.5	9.4	11	77	79
Mean ± SE	2.2 ± 0.3	2.2 ± 0.4	12 ± 1	12 ± 1	76 ± 5	81 ± 5

*Mean values based on plasma mass determined immunoturbidimetrically on fasting specimens obtained daily during first 4 days of each turnover study.

†Mean values based on plasma mass determined by isopropanol precipitation on fasting specimens obtained daily during first 4 days of each turnover study.[42]

^{131}I-labeled VLDL during HRT versus control conditions for a representative subject are shown in Fig 1. Kinetic parameters of VLDL turnover calculated from ^{131}I-labeled VLDL apo B decay curves are listed in Table 4. During the treatment versus control periods, there were no significant changes in FCR, production rate, or pool size of VLDL apo B ($P > .4$).

Kinetic parameters of LDL turnover calculated from ^{125}I-labeled LDL decay curves are listed in Table 5. FCR of LDL apo B was increased significantly by an average of 13% ± 4% during HRT versus control (0.34 ± 0.03 *v* 0.30 ± 0.02 pools/d, $P < .05$). Figure 2 illustrates the effect of HRT on the percent decrease of ^{125}I-labeled LDL apo B specific activity from the plasma for a representative subject. Enhancement of LDL apo B catabolism was not accompanied by any systematic change in pool size of LDL apo B, because LDL apo B production rate also tended to increase, although not significantly, during HRT versus control (14 ± 0.5 *v* 13 ± 0.9 mg/kg/d, $P > .1$).

Evaluation of relationships[53] between lipoprotein fractions indicated that VLDL apo B was the sole precursor of IDL apo B (Fig 1). Comparisons of the areas under specific-activity curves for LDL apo B and IDL apo B, as described by Goldberg et al,[47] indicated that LDL production derived from plasma VLDL via IDL accounted for a similar proportion of total LDL apo B production during HRT versus control (30% *v* 29%, respectively). This is consistent with a substantial portion of LDL entering the circulation directly[47] and/or via small, rapid-turnover, hepatogenous pool(s) of VLDL apo B,[54] which is not traceable with exogenous iodine labeling.[42] The present experiments could not distinguish relative contributions of the latter pathways.

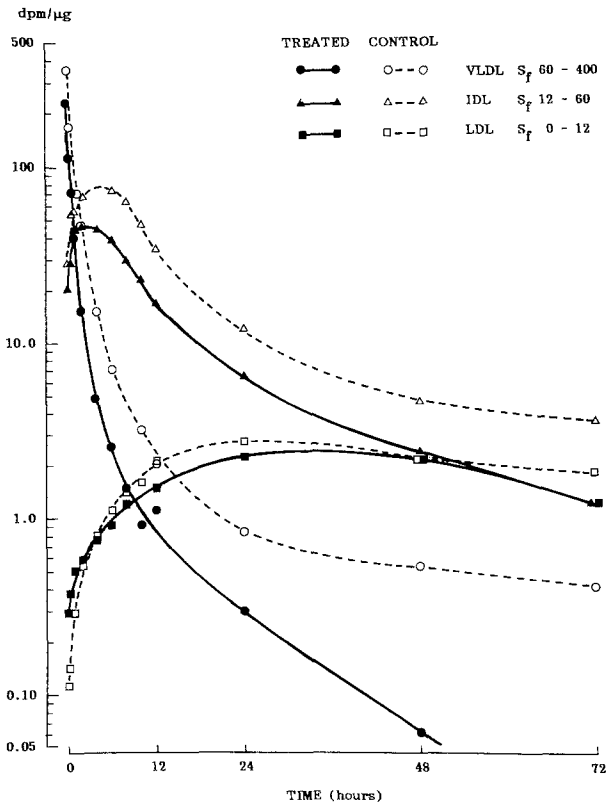

Fig 1. Specific activities of apo B of VLDL, IDL, and LDL after intravenous injection of autologous ^{131}I-labeled VLDL during control period and HRT for a representative subject.

Table 4. Kinetic Parameters of Human ^{125}I-VLDL Apo B Turnover in HRT-Treated Postmenopausal Subjects

Subject No.	FCR (pools/d)	Pool Size of VLDL Apo B (mg/kg)*	Production Rate (mg/kg/d)†
1			
Control	13	1.5	20
Treated	17	2.0	34
2			
Control	5.0	2.6	13
Treated	5.6	2.6	15
3			
Control	2.7	4.8	13
Treated	2.4	5.4	13
4			
Control	5.9	4.1	25
Treated	6.1	2.4	15
5			
Control	9.7	2.3	22
Treated	8.1	1.4	12
6			
Control	12	1.7	20
Treated	8.6	1.2	10
Mean ± SE			
Control	8.1 ± 1.7	2.8 ± 0.6	19 ± 2
Treated	8.0 ± 2.0	2.5 ± 0.6	16 ± 4

*Based on kinetic analysis of ^{131}I-labeled VLDL apo B.

†Based on product of FCR and radioisotopic determination of VLDL apo B pool size.

DISCUSSION

The present studies are the first to investigate effects on composition and turnover of VLDL and LDL that result from administration of this recently recommended regimen of HRT.[32] The 10% ± 2% mean reduction in the mean plasma concentration of lipoproteins of S_f 0-20 during HRT in the present short-term study is similar to that observed during a 1-year treatment with a similar HRT regimen in a much larger group of subjects.[26] Short-term studies using CEE 0.625 mg/d alone have also shown similar reductions in LDL cholesterol.[55-58] The present study shows that HRT alters the composition of LDL (S_f 0-12) by significantly reducing its cholesterol content. The trend toward an enrichment of triglyceride content of LDL (S_f 0-12) at the expense of cholesterol with the present HRT is consistent with changes observed during daily administration of CEE 0.625 mg alone[57-59]; however, the effect on LDL particle size was not determined in the present study. Treatment with estrogen alone has also been associated with a decrease in the proportion of large LDL particles and an increase in the proportion of smaller LDL particles in postmenopausal women.[60,61] This has been more pronounced in subjects with a preponderance of large LDL particles at baseline.[60] Low intakes of saturated fat and cholesterol, widely used to

Table 5. Kinetic Parameters of Human ^{131}I-LDL Apo B Turnover in HRT-Treated Postmenopausal Subjects

Subject No.	FCR (pools/d)	LDL Apo B (mg/kg)	Production Rate (mg/kg/d)			Fraction of LDL Apo B Production Derived From IDL Apo B (%)*
			Total	Direct	Indirect	
1						
Control	0.23	42	9.6	6.4	3.2	33
Treated	0.27	50	13	7.7	5.4	41
2						
Control	0.36	30	11	8.5	2.4	22
Treated	0.43	28	12	9.1	2.9	24
3						
Control	0.25	48	12	10	2.2	18
Treated	0.28	48	14	11	2.2	16
4						
Control	0.34	45	15	9.7	5.7	37
Treated	0.43	36	15	12	3.5	23
5						
Control	0.31	49	15	9.9	5.4	35
Treated	0.29	52	15	12	3.6	24
6						
Control	0.31	42	13	9.2	3.8	29
Treated	0.36	36	13	7.0	5.7	45
Mean ± SE						
Control	0.30 ± 0.02	43 ± 3	13 ± 0.9	9.0 ± 0.5	3.8 ± 0.6	29 ± 3
Treated	0.34 ± 0.03†	42 ± 4	14 ± 0.5	9.8 ± 0.9	3.9 ± 0.6	29 ± 5

*Calculated according to method reported by Goldberg et al.[47]

†Different from control, $P < .04$.

decrease the risk for CHD, have also been associated with reductions in LDL particle size. Hence, a small LDL particle size appears not to be a good indicator of atherosclerotic risk, at least in population studies.[62]

Despite the change in lipoprotein composition with the present HRT, there was no change in apo B pool size of LDL (S_f 0-12; Table 4). HRT appeared to induce a change in the compositional pattern of lipoprotein particles of S_f 0-12; however, the present experimental design did not allow for analysis of LDL subfractions, and heterogeneity within LDL subfractions could obscure metabolic changes occurring in response to treatment. Although an increase in direct synthesis of LDL could conceivably result in changes in LDL composition, we found no statistically significant increase in LDL production via this pathway, although there were increases in five of six subjects (Table 5). HRT, like obesity,[63] increased FCR of LDL (Table 5); however, this has not been related to alteration of particle size in humans. While we acknowledge the uncertainty about the effect of gender on LDL kinetics, it is noteworthy that the mean control value for total production rate of LDL apo B obtained in women by the present kinetic analysis was similar to that obtained in non-obese men by multicompartmental analysis.[64]

HRT induced increases in mean FCR of LDL apo B and total LDL apo B production (Table 4) that were less than half those reported with estrogen alone.[58] Some differences between these results may be accounted for by differences between subjects studied and estrogen type and dosage (CEE 0.625 mg *v* estradiol-17β 2 mg) or by the present addition of low-dosage MPA, a C-21 progestin. The proges-

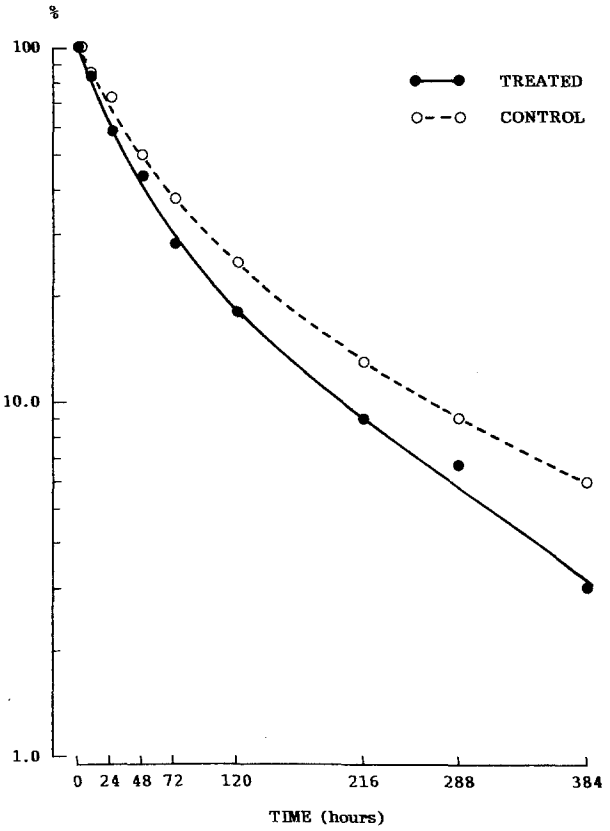

Fig 2. Specific radioactivity of LDL apo B expressed as % of peak specific activity after intravenous injection of autologous ^{125}I-labeled LDL for a representative subject.

tin effect on lipids has been related to the type of progestin (C-21 *v* C-19).[65] The present HRT decreased neither the pool size nor the production rate of LDL apo B, whereas continuous low-dosage DL-norgestrel (0.075 mg/d), a C-19 progestin, combined with estradiol-17β 1 mg (25 of 28 days) decreased both LDL production and LDL pool size.[36]

Although continuous administration of high-dose MPA (10 mg/d) abolishes the increment in HDL cholesterol induced by a daily dose of CEE 0.625 mg,[24] continuous administration of daily low-dose MPA (2.5 mg) combined with CEE 0.625 mg preserves the beneficial effects of CEE on plasma lipoproteins. The findings of a significant (16%) increase in HDL cholesterol and a significant (10%) decrease in LDL cholesterol during the present short-term HRT with MPA + CEE are consistent with results obtained in studies of 1 year's duration.[26,66] The reported mean increments in HDL cholesterol with MPA + CEE range from 6% to 16%, versus 7% to 19% with CEE 0.625 mg alone.[57-59,67] Mean reductions in LDL cholesterol with MPA + CEE range from 10% to 29%, versus 15% to 16% with CEE alone.[57-59,67]

The addition of progestin to estrogen may have a beneficial effect on VLDL metabolism by modulating the action of estrogen thereon. Whereas administration of estradiol alone in another study of postmenopausal women increased VLDL apo B production, VLDL pool size, and plasma triglyceride concentration,[58] the present combined low-dose CEE + MPA (C-21 progestin) had no apparent adverse effects on VLDL metabolism (Table 4). The nature of the progestin (C-19 *v* C-21) coadministered with the progestin also appears to influence the effect of the estrogen on VLDL metabolism. Whereas MPA + CEE had no significant effect on metabolic parameters of VLDL metabolism (Table 4), combined administration of low-dosage DL-norgestrel (C-19 progestin) and estradiol-17β to postmenopausal women decreases VLDL pool size and plasma total triglyceride concentration by increasing FCR of VLDL.[36]

Both the increase in HDL cholesterol and the decrease in cholesterol content of LDL underlie significant reductions in mean values for the ratios plasma total cholesterol to HDL cholesterol and LDL (S_f 0-20) cholesterol to HDL cholesterol from ≥ 75th percentile (ie, 5.2 and 3.5, respectively) during the control period to near the 50th percentile (ie, 4.2 and 2.7, respectively[68]) during HRT. Estrogen-induced increases in HDL cholesterol concentration have been attributed to increased synthesis of apo A1[69] and/or reduced catabolism.[70]

Thyroid hormone influences lipid metabolism, but long-term use in a physiologic dose for thyroid replacement in subject no. 2 would not be expected to perturb lipid metabolism. Hydrochlorothiazide can increase serum cholesterol[71]; however, any potential effect on lipid metabolism in subjects no. 4 and 6, who were treated with hydrochlorothiazide, was minimized by maintaining the same dosage during both control and treatment periods of each experiment. The sample size of the present study was too small to investigate a possible effect of hydrochlorothiazide; nevertheless, there was no indication of any such effect. To eliminate the contribution of intestinal lipoproteins to plasma triglyceride-rich lipoproteins during the period of frequent sampling on day 1, fat intake was reduced to less than 5 g/d.[35] The same dietary protocol was used during both the control and experimental periods of the study to minimize any potential effect on lipoprotein metabolism. In addition, lipoprotein concentrations remained steady throughout the turnover study.

In summary, the present combined low-dose MPA + CEE regimen significantly altered the composition and fractional catabolism of LDL and favorably influenced the overall plasma lipoprotein lipid vascular risk profile.[72]

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