Effects of continuous conjugated estrogen and micronized progesterone therapy upon lipoprotein metabolism in postmenopausal women

Bernard M. Wolfe,[1,*] P. Hugh R. Barrett,[†] Lisanne Laurier,[*] and Murray W. Huff[*]

Department of Medicine,[*] The University of Western Ontario, London, Ontario, Canada, N6A 5A5
and Department of Medicine,[†] University of Western Australia, Perth, WA 6000, Australia

Abstract **The effects of continuously administering both conjugated equine estrogens (CEE) and micronized progesterone (MP) on the concentration, composition, production and catabolism of very low density (VLDL) and low density lipoproteins (LDL) have not previously been reported. The mechanism of the hormonally induced reductions of plasma LDL cholesterol of S_f 0–20 (mean 16%, $P < 0.005$) and LDL apoB (mean 6%, $P < 0.025$) were investigated by studying the kinetics of VLDL and LDL apolipoprotein (apo) B turnover after injecting autologous ^{131}I-labeled VLDL and ^{125}I-labeled LDL into each of the 6 moderately hypercholesterolemic postmenopausal subjects under control conditions and again in the fourth week of a 7-week course of therapy (0.625 mg/d of CEE + 200 mg/d of MP). The combined hormones significantly lowered plasma LDL apoB by increasing the mean fractional catabolic rate of LDL apoB by 20% (0.32 vs. 0.27 pools/d, $P < 0.03$). Treatment also induced a significant increase in IDL production (6.3 vs. 3.7 mg/kg/d, $P = 0.028$). However, this did not result in an increase in LDL production because of an increase in IDL apoB direct catabolism (mean 102%, $P = 0.033$). VLDL kinetic parameters were unchanged and the concentrations of plasma total triglycerides (TG), VLDL-TG, VLDL-apoB did not rise as often seen with estrogen alone. Plasma HDL-cholesterol rose significantly ($P < 0.02$).** **Our major conclusion is that increased fractional catabolism of LDL underlies the LDL-lowering effect of the combined hormones.**—Wolfe, B. M., P. H. R. Barrett, L. Laurier, and M. W. Huff. **Effects of continuous conjugated estrogen and micronized progesterone therapy upon lipoprotein metabolism in postmenopausal women.** ***J. Lipid Res.*** **2000.** 41: **368–375.**

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Ovarian hormone replacement therapy, defined as treatment with estrogen and progestin (HRT), provides effective relief of vasomotor symptoms (1), prevents osteoporosis (2) and may reduce coronary heart disease (CHD, 3). Although a recent randomized clinical trial in older postmenopausal (PMP) women with advanced CHD including coronary bypass surgery found no overall cardiovascular benefit of HRT (4), observational studies of HRT or replacement of estrogen alone (ERT) have reported reductions in both all-cause and CHD mortality in other PMP women (5, 6).

Elevations of plasma cholesterol (7, 8) and triglycerides (7) have been reported in a high proportion of PMP women and are thought to contribute to the associated increase in CHD (3, 9–11). Estrogen replacement by HRT or ERT has the potential to reduce CHD risk through favorable effects on both lipoproteins and the arterial wall (12). Increases in both plasma triglycerides and HDL cholesterol have been reported in response to ERT in PMP women (13). However, triglyceride responses to HRT involving continuous conjugated estrogen (CEE, 0.625 mg/d) and medroxyprogesterone acetate (MPA, 2.5 mg/d) have been inconsistent. Significant increases in triglycerides have been reported in some, but not all, studies of 12 months or more in duration (14–17), but no significant increase in plasma triglycerides has been reported in shorter studies of 1.7 to 9 months in duration (18–23). MPA has also been reported to significantly reduce the CEE-induced increase in plasma triglycerides (15). Furthermore, estrogen-induced increments in HDL cholesterol have tended to be blunted more by concomitant administration of MPA than by natural ovarian progestin administered as micronized progesterone (MP 14, 24). Because prolonged estrogen therapy that is unopposed by progestin is associated with risk of endometrial neoplasia, there is a need for progestin (14, 25, 26).

Despite clinical usage of MP together with CEE by PMP women (14, 24), there has been no information about the

Abbreviations: CHD, coronary heart disease; CEE, conjugated equine estrogen; MP, micronized progesterone; FCR, fractional catabolic rate; HRT, hormonal replacement therapy; IDL, intermediate density lipoproteins; MPA, medroxyprogesterone acetate; Lp[a], lipoprotein [a].

[1] To whom correspondence should be addressed.

effects of continuously administering CEE and MP (CEE_{cont}/MP_{cont}) on the concentrations, production, catabolism and/or composition of the major apolipoprotein (apo) B-containing lipoproteins [very low density (VLDL), intermediate density (IDL) and low density lipoproteins (LDL)] in PMP women. The present study was undertaken to test the hypothesis that HRT with CEE_{cont}/MP_{cont} would lower LDL-cholesterol by enhancing its fractional catabolism and would blunt the increases in VLDL production.

SUBJECTS AND METHODS

Subjects

Six participants, who had experienced typical menopausal symptoms and were amenorrheic for at least 6 months (serum follicle stimulating hormone >70 U/L) were recruited from London Health Sciences Centre University Campus out-patient clinics (**Table 1**). Baseline fasting plasma cholesterol concentrations during Phase I American Heart Association diets prior to entry into the study exceeded 5.2 mmol/L, the cut-point of eligibility for dietary treatment recommended by the National Cholesterol Education Program (27). Two subjects had fasting levels of plasma HDL cholesterol levels which were below the recommended cut-point of 0.9 mmol/L (27), 2 had plasma triglycerides which exceeded the desirable upper limit of 2.3 mmol/L (28), and 2 had fasting levels of lipoprotein [a] (Lp[a]) which were slightly above the 95th percentile (29). One subject had primary hypothyroidism and was treated throughout the control and experimental periods with l-thyroxine 0.15 mg/d, along with 50 mg of captopril and 50 mg of hydrochlorothiazide daily for hypertension. Although hydrochlorothiazide can raise serum cholesterol (30), any potential effect on lipid metabolism was minimized by maintaining the same dosage level during both control and treatment periods of the experiment. Subjects received no other medications known to affect lipid metabolism. Intake of ethanol was less than 30 ml/d for the month before each study and was disallowed during the turnover studies. Subjects were instructed to maintain their low fat diets and their regular physical activity for the duration of the study, and body weight was monitored on eight occasions during each control and treatment period of their studies. Thus, mean values for body weight (Table 1) and body mass index (23.7 ± 1.3 vs. 23.6 ± 1.3) were similar during the control and treatment periods ($P >$ 0.5). All subjects had normal fasting concentrations of serum glucose, thyroxine, creatinine, calcium, albumin, sodium, potassium, chloride, and bicarbonate. Hematological and urine analyses were normal. The experimental protocol 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 containing approximately 29% of energy from fat (mean ratio of polyunsaturated:monounsaturated:saturated fat ca 0.8:1.3:1.0, 200 mg cholesterol per day), 19% of energy from protein, and 52% of energy from carbohydrate. During admissions to hospital for the first day only of each turnover study the diet was unchanged, except that the carbohydrate content of the diet was increased at the expense of fat to maintain caloric intake. Fat intake was reduced to <5 g per day to essentially eliminate the contribution of intestinal lipoproteins to plasma triglyceride-rich lipoproteins (31). Samples of venous blood for determination of plasma total cholesterol, LDL cholesterol (S_f 0–20) and HDL cholesterol, total triglycerides, and Lp[a] concentrations were obtained from a forearm vein (after a 12-h fast) from each subject on four occasions at approximately weekly intervals *a*) during the initial 3-week control period before starting hormonal replacement and *b*) during weeks 3 to 6 of the 7-week course of treatment with CEE (0.625 mg/d) and MP (200 mg/d), together referred to as CEE_{cont}/MP_{cont}. Subjects underwent studies of the kinetics of VLDL and LDL apoB turnover during the control period and again during the last 16 days of hormone replacement therapy (HRT) with CEE_{cont}/MP_{cont}. All subjects received 300 mg/d potassium iodide for 3 days before and 13 days after the reinjection of their radiolabeled lipoproteins.

Preparation of labeled lipoproteins and kinetic studies

The procedures for the lipoprotein turnover studies, including the isolation and preparation of labeled lipoproteins and their reinjection, have previously been described, along with the sampling and fractionation of VLDL (S_f 60–400), intermediate density lipoprotein (IDL, S_f 12–60), and LDL (S_f 0–12) and the isolation of apoB from each lipoprotein fraction by isopropanol precipitation and determination of its specfic activity (23, 32–35). The bolus injection of ^{131}I-labeled VLDL was immediately followed by the ^{125}I-labeled LDL. Isotopic crossover was corrected throughout the studies. Lipids were measured in these lipoprotein fractions as described below.

TABLE 1. Characteristics of postmenopausal subjects

			Weight		Cholesterol		Triglycerides		HDL-cholesterol	
Subject	Age	Height	Control	Treated	Control	Treated	Control	Treated	Control	Treated
	yr	*cm*	*kg*		*mmol/L*		*mmol/L*		*mg/dl*	
1	58	164	50	50	6.70	6.59	1.65	2.24	0.98	1.23
2	79	152	62	60	6.10	5.09	1.27	1.36	1.28	1.35
3	72	151	50	50	7.22	6.80	1.27	1.27	1.22	1.48
4	46	158	59	59	6.59	6.41	5.83	3.99	0.58	0.95
5	69	161	62	63	6.21	5.79	2.33	1.84	1.03	1.53
6	57	159	71	71	6.30	5.60	1.57	1.47	1.30	1.45
Mean	64	158	59	59	6.57	6.05[a]	2.32	2.03	1.07	1.33[b]
± SE	5	2	3	3	0.19	0.27	0.72	0.42	0.11	0.09

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

[a] Significantly different from control, $P < 0.01$.

[b] Significantly different from control, $P < 0.025$.

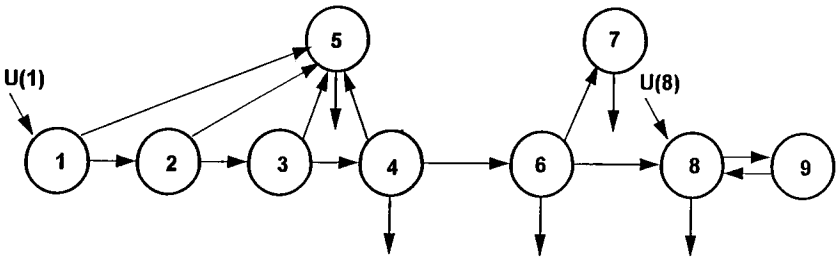

Fig. 1. Multicompartmental model for apoB metabolism. See Methods: Kinetic Analysis for details.

Kinetic analysis

A multicompartmental model was used to describe VLDL, IDL, and LDL-apoB tracer data. In multicompartmental modeling, each compartment or pool represents a group of kinetically homogeneous particles. In this study the SAAM II program (SAAM Inst., Seattle, WA) was used to fit the model to the observed tracer data. ApoB metabolic parameters are subsequently derived from the model parameters giving the best fit. All tracer data was fit simultaneously using the compartmental model (**Fig. 1**).

Compartments 1 through 5 are used to describe the kinetics of apoB in the VLDL fraction. It was assumed that all VLDL apoB enters plasma via compartment 1. Compartments 1 though 4 represent a delipidation chain or cascade as originally described by Phair et al. (36). It is assumed that the residence time of particles in each compartment of the chain is equal. In addition, the fraction of each compartment in the cascade converted to the slowly turning over VLDL compartment, compartment 5, is the same. VLDL particles in compartment 4 can be converted to IDL or can be removed directly from plasma. The IDL section of the model includes compartments 6 and 7, a rapidly and slowly turning over pool of IDL particles, respectively. Particles in compartment 6 can be converted to the slow IDL compartment, to LDL, or can be removed directly from plasma. Because labeled IDL was not injected, it was assumed that all IDL was derived from VLDL. The LDL section of the model is characterized by a plasma compartment, compartment 8, and an extravascular exchange compartment, compartment 9. This model assumes that LDL apoB is kinetically homogeneous; however, the isolation of LDL subfractions and/or the collection of urine radioactivity, after the injection of labeled LDL, may provide evidence of kinetic heterogeneity within the LDL fraction. Examination of the raw data revealed that significant input of "cold" or unlabeled LDL must occur, as seen by observing the relationship between the ^{131}I-labeled IDL and LDL apoB specific radioactivity curves. The present model puts quantitative values on these observations. The model that was fit to the experimental data was the simplest model consistent with the data. Errors of parameters were low (<20%) and the residuals were randomly allocated around the model fits.

Analyses of metabolites

Plasma VLDL and IDL apoB concentrations were measured immunoturbidimetrically using Tina-quant apolipoprotein B Kit obtained from Boehringer Mannheim Canada, Laval, Quebec. The assay was standardized to LDL isolated by ultracentrifugation (d 1.040–1.063) in which it was determined that apoB was the only protein present. The protein was determined by the modified Lowry procedure (37). Lp[a] was measured using Macra Lp[a] Kit obtained from Terumo Medical Corp., Elkton, MD. The intra-assay coefficients of variation for apoB and Lp[a] were 1.8 and 4.7%, respectively.

Fasting venous blood samples were also obtained at weekly intervals from weeks 3–6 for measurement of lipids, including LDL cholesterol of S_f 0–20, which was determined at these time points by subtraction of the cholesterol content of VLDL of S_f 20–400 (38) plus HDL cholesterol from that of whole plasma. Concentrations of cholesterol and triglycerides in the chloroform–methanol extract of whole plasma were determined as previously described (39), except that diagnostic kits from Boehringer Mannheim GmbH Diagnostica, Montreal, Quebec (C-system Kit for cholesterol and Test Combination Kit for triglycerides) were used to measure cholesterol content of fasting plasma VLDL, IDL, and LDL and triglyceride content of VLDL during the last 16 days the turnover studies because of the small amounts of material available for assay. HDL cholesterol was measured after precipitation with heparin–manganese chloride (40).

Values from control and treatment periods were compared using Student's paired two-tailed *t*-test (41) or the Wilcoxon signed rank test (42), as specified. Variance was expressed as standard error of the mean.

RESULTS

Concentrations of lipoprotein lipids and apoB

During weeks 3 to 6 of the administration of CEE_{cont}/MP_{cont}, there were significant decreases of 8 ± 2% in fasting plasma total cholesterol (6.05 ± 0.27 vs. 6.57 ± 0.19 mmol/L, $P < 0.01$, Table 1) and 16 ± 2% in the concentration of cholesterol of LDL of S_f 0–20 (4.13 ± 0.21 vs. 4.86 ± 0.21 mmol/L, $P < 0.005$)[3], whereas HDL cholesterol rose by 30 ± 9% ($P < 0.025$, Table 1). The hormones induced a 29 ± 3% decrease in the mean value for the ratio of total cholesterol to HDL cholesterol versus control (4.6 ± 0.5 vs. 6.6 ± 1.0, $P < 0.02$) and a 35 ± 2% decrease in the value of the ratio of LDL (S_f 0–20) cholesterol to HDL cholesterol (3.1 ± 0.3 vs. 4.9 ± 0.7, $P < 0.01$).

Data obtained at time of the turnover studies comparing control versus treatment indicated that CEE_{cont}/MP_{cont} reduced the mean plasma concentration of cholesterol transported in LDL of S_f 0–12 by 13 ± 3% (3.70 ± 0.24 vs. 4.27 ± 0.19 mmol/L, $P < 0.005$) and increased mean HDL cholesterol by 30 ± 8% (1.32 ± 0.10 vs. 1.01 ± 0.09 mmol/L, $P < 0.01$, **Table 2**). Simultaneously, the mean values tended to decline during treatment for each of plasma total triglycerides (1.72 ± 0.36 vs. 2.20 ± 0.34 mmol/L), VLDL triglycerides (0.75 ± 0.17 vs. 0.96 ± 0.20 mmol/L, Table 2), IDL triglycerides (0.52 ± 0.16 vs. 0.69 ± 0.10 mmol/L), and LDL triglycerides (0.28 ± 0.02 vs. 0.36 ± 0.056 mmol/L), however, the changes were not

[3] LDL cholesterol of S_f 0–20 was not determined during turnover studies when LDL of S_f 0–12 was measured.

TABLE 2. Effects of CEE_{cont}/MP_{cont} on levels of cholesterol and triglycerides of plasma lipoproteins on first day of turnover studies

Subject, Period	Whole Plasma		VLDL		IDL	LDL	HDL
	Cholesterol	Triglycerides	Cholesterol	Triglycerides	Cholesterol	Cholesterol	Cholesterol
	mmol/L						
1							
Control	6.31	1.85	0.18	0.81	0.70	4.63	0.80
Treated	6.52	2.44	0.22	1.09	0.78	4.34	1.19
2							
Control	5.30	1.17	0.08	0.51	0.29	3.75	1.19
Treated	4.81	1.27	0.17	0.60	0.29	2.95	1.40
3							
Control	6.90	2.06	0.23	0.72	0.72	4.94	1.01
Treated	6.52	0.78	0.09	0.28	0.44	4.47	1.50
4							
Control	6.80	3.42	0.52	1.72	1.14	4.45	0.70
Treated	6.10	3.13	0.34	1.41	1.40	3.47	0.91
5							
Control	6.21	2.93	0.34	1.42	0.85	3.91	1.11
Treated	5.79	1.27	0.15	0.51	0.36	3.67	1.60
6							
Control	5.71	1.74	0.10	0.59	0.47	3.95	1.27
Treated	5.19	1.45	0.08	0.59	0.52	3.31	1.32
Mean ± SE							
Control	6.21 ± 0.25	2.20 ± 0.34	0.24 ± 0.07	0.96 ± 0.20	0.70 ± 0.12	4.27 ± 0.19	1.01 ± 0.09
Treated	5.82 ± 0.29[a]	1.72 ± 0.36	0.18 ± 0.04	0.75 ± 0.17	0.63 ± 0.17	3.70 ± 0.24[b]	1.32 ± 0.10[c]

Values for each subject were based upon fasting blood plasma samples obtained immediately prior to injection of autologous labeled ^{131}I-labeled VLDL (S_f 60–400) and ^{125}I-labeled LDL (S_f 0–12) at the beginning of each turnover study.

[a] Significantly different from control, $P < 0.05$.
[b] Significantly different from control, $P < 0.005$.
[c] Significantly different from control, $P < 0.01$.

statistically significant. The changes in the mean values for concentrations of HDL triglycerides (0.17 ± 0.02 vs. 0.19 ± 0.02 mmol/L) and Lp[a] (17 ± 8 vs. 20 ± 10 mg/dl) also failed to be statistically significant. The mean value for concentration of apoB in plasma LDL was significantly lower during hormonal treatment versus control (82 ± 4 vs. 87 ± 3 mg/dl, $P < 0.05$); however, there was no significant change in mean VLDL apoB concentration (5.1 ± 0.9 vs. 4.3 ± 0.5 mg/dl, $P > 0.2$).

The mean value for the ratio of cholesterol to apoB in plasma VLDL (S_f 60–400) was 27 ± 4% lower during hormonal treatment versus control (1.5 ± 0.6 vs. 2.1 ± 0.9, $P < 0.02$ by Wilcoxon signed rank test), but there was no change in the ratio of triglycerides to apoB (16 ± 6 vs. 17 ± 5, $P > 0.5$). The mean value for the ratio of cholesterol to apoB in plasma IDL (S_f 12–60) was significantly lower (by 17 ± 2%) during CEE_{cont}/MP_{cont} versus control (1.8 ± 0.1 vs. 2.2 ± 0.2, $P < 0.005$), but there was no change in the ratio of triglycerides to apoB in IDL (4.0 ± 0.6 vs. 4.1 ± 0.5, respectively, $P > 0.5$). There was no significant change in the mean value for the ratio of cholesterol to apoB of plasma LDL (S_f 0–12) during CEE_{cont}/MP_{cont} versus control (1.4 ± 0.1 vs. 1.5 ± 0.1, respectively, $P > 0.1$), nor in the ratio of triglyceride to apoB (0.29 ± 0.02 vs. 0.27 ± 0.03, $P > 0.3$).

Consistent with a steady-state, there were no systematic changes during the turnover studies in plasma concentrations of the most readily quantified indices, namely total protein content of LDL (mean coefficient of variation of 10.7%, corresponding to a mean SD of 9.2 mg/dl, n = 6 paired studies) and total protein content of VLDL (mean coefficient of variation of 23%, corresponding to a mean SD of 1.7 mg/dl, n = 6 paired studies).

Metabolism of lipoproteins

Values for the specific activities of ^{131}I-labeled apoB in VLDL, IDL, and LDL over 72 h after injection of ^{131}I-labeled VLDL during CEE_{cont}/MP_{cont} versus control for a representative subject are shown in **Fig. 2**. The kinetic parameters of apoB were determined from the simultaneous analysis of all the specific activity data by using the model shown in Fig. 1. The kinetic parameters of VLDL turnover are summarized in **Table 3**. There were nonsignificant trends during treatment with CEE_{cont}/MP_{cont} towards increases in production rate of VLDL apoB (mean values 11.4 ± 2.1 vs. 9.4 ± 1.6 mg/kg/d, $P = 0.26$), in fractional catabolic rate of VLDL apoB (mean values 5.2 ± 0.8 vs. 4.7 ± 0.5, $P = 0.47$) and in pool size of VLDL apoB (133 ± 20 vs. 113 ± 9 mg, $P > 0.2$). That the conversion of VLDL to LDL did not change (36 ± 6 vs. 33 ± 6%, $P > 0.4$) despite increased conversion of VLDL to IDL (62 ± 7 vs. 45 ± 11%, $P = 0.05$) is consistent with a greater proportion of the IDL pool being cleared directly and not converted to LDL. IDL to LDL conversion decreased during treatment (74 ± 5 vs. 87 ± 5, $P = 0.033$) indicating that a higher proportion of IDL was cleared directly (26 vs. 13%). Sig-

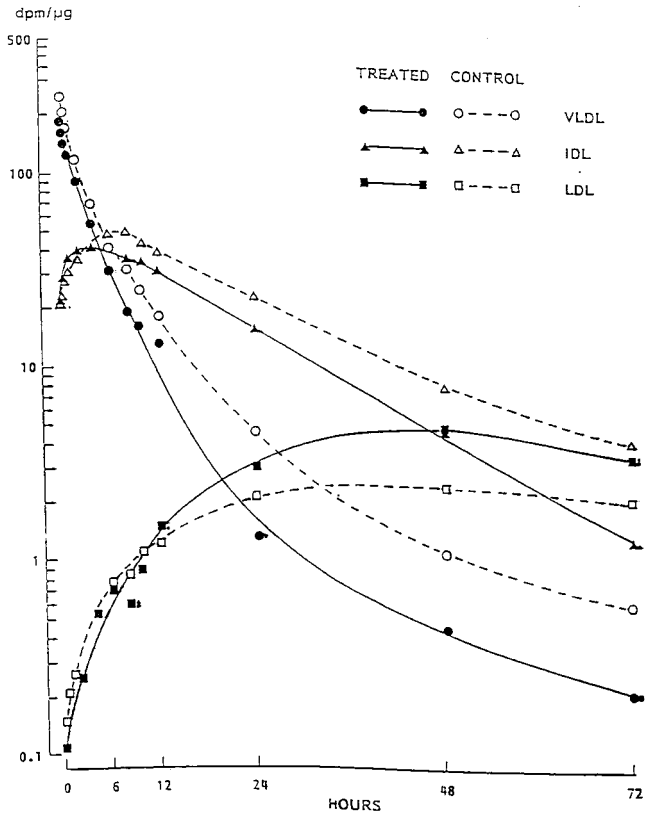

Fig. 2. Specific activity–time curves of apoB of VLDL, ILD, and LDL after intravenous injection of autologous ^{131}I-labeled VLDL during control (open symbols) and conjugated equine estrogen/micronized progesterone treatment (closed symbols) for subject no. 1.

nificantly increased conversion of VLDL to IDL during hormone administration partly explains the significant increase in IDL apoB production (6.3 ± 0.5 vs. 3.7 ± 0.8 mg/kg/d, $P = 0.028$, **Table 4**). The increased IDL production would more than offset the trend towards an increase in IDL FCR (1.55 ± 0.29 vs. 1.00 ± 0.11 pools/d, $P = 0.086$), such that there was no significant change in IDL apoB pool size (294 ± 59 vs. 229 ± 42 mg, $P > 0.4$).

The kinetic parameters of LDL turnover are summarized in **Table 5**. The FCR of LDL apoB increased significantly, by an average of 20 ± 7%, during hormonal administration versus control (0.320 ± 0.021 vs. 0.269 ± 0.016 pools/d, $P = 0.03$). This resulted in a significant 6 ± 2% decrease in LDL apoB pool size (2162 ± 173 vs. 2300 ± 149 mg, $P < 0.05$). Although production of LDL apoB tended to rise, with increases in 5 of the 6 subjects, the change was not significant ($P = 0.10$). LDL direct synthesis was not significantly altered by the combined hormones (8.3 ± 1.5 vs. 7.8 ± 0.9 mg/kg/d, $P = 0.58$) and direct LDL production as a percent of total LDL production was unchanged by hormonal treatment (67 ± 7 vs. 73 ± 4%, $P = 0.35$).

Figure 3 illustrates the effect of CEE_{cont}/MP_{cont} on the decrease of the ^{125}I-labeled LDL apoB specific activity from the plasma for a representative subject. Data in Fig. 3 are presented as percent of peak specific activity because absolute values for peak specific activity were different between the treatment and control. The steeper slope during treatment indicates that the fractional catabolic rate of LDL is more rapid during hormonal treatment than control.

TABLE 3. Kinetic parameters of human VLDL apoB turnover in CEE_{cont}/MP_{cont}-treated postmenopausal subjects

Subject, Period	VLDL Pool Size	Fractional Catabolic Rate	VLDL Total Production	Conversion of VLDL to IDL	Conversion of VLDL to LDL
	mg	*pools/d*	*mg/kg/d*	*% production*	
1					
Control	140	4.8	13.5	24	20
Treated	214	3.6	15.3	47	15
2					
Control	92	5.5	8.2	41	35
Treated	138	7.5	17.3	43	30
3					
Control	92	4.8	8.9	36	35
Treated	68	6.5	8.8	57	49
4					
Control	101	2.5	4.3	98	61
Treated	101	2.0	3.4	93	56
5					
Control	142	6.4	14.7	40	22
Treated	140	6.0	13.4	61	26
6					
Control	112	4.3	6.9	32	23
Treated	134	5.4	10.2	69	38
Mean ± SE					
Control	113 ± 9	4.7 ± 0.5	9.4 ± 1.6	45 ± 11	33 ± 6
Treated	133 ± 20	5.2 ± 0.8	11.4 ± 2.1	62 ± 7[a]	36 ± 6

[a] Significantly different from control, $P < 0.05$.

TABLE 4. Kinetic parameters of human IDL apoB turnover in CEE_{cont}/MP_{cont}-treated postmenopausal subjects

Subject, Period	IDL Pool Size	Fractional Catabolic Rate	IDL Total Production
	mg	*pools/d*	*mg/kg/d*
1			
Control	173	0.93	5.8
Treated	256	1.39	8.2
2			
Control	145	1.47	3.4
Treated	199	2.21	7.4
3			
Control	198	0.80	3.2
Treated	112	2.24	5.0
4			
Control	335	0.74	4.2
Treated	520	0.36	3.2
5			
Control	173	0.95	3.2
Treated	256	1.79	7.1
6			
Control	142	1.12	2.2
Treated	393	1.28	7.1
Mean ± SE			
Control	229 ± 42	1.00 ± 0.11	3.7 ± 0.5
Treated	294 ± 59	1.55 ± 0.29	6.3 ± 0.8[a]

[a] Significantly different from control, $P = 0.028$.

TABLE 5. Kinetic parameters of human LDL apoB turnover in CEE_{cont}/MP_{cont}-treated postmenopausal subjects

Subject, Period	LDL Pool Size	Fractional Catabolic Rate	LDL Total Production	LDL Direct Production	
	mg	*pools/d*	*mg/kg/d*	*mg/kg/d*	*%*
1					
Control	2093	0.224	10.0	6.8	68
Treated	1913	0.313	11.8	8.4	71
2					
Control	2037	0.234	7.7	4.8	63
Treated	1701	0.313	8.9	3.6	41
3					
Control	2070	0.261	10.8	7.7	71
Treated	1935	0.232	9.0	4.6	51
4					
Control	2336	0.335	13.3	10.7	80
Treated	2257	0.391	15.0	13.0	87
5					
Control	2260	0.274	9.4	6.7	71
Treated	2260	0.324	12.0	9.8	81
6					
Control	3003	0.284	12.0	9.8	87
Treated	2907	0.349	14.3	10.4	73
Mean ± SE					
Control	2300 ± 149	0.269 ± 0.016	10.8 ± 0.8	7.8 ± 0.9	73 ± 4
Treated	2162 ± 173[a]	0.320 ± 0.021[b]	11.8 ± 1.0	8.3 ± 1.5	67 ± 7

[a] Significantly different from control, $P < 0.05$.
[b] Significantly different from control, $P = 0.03$.

DISCUSSION

The present studies are the first to determine the effects of the continuously administered CEE and MP on the production, catabolism, and composition of VLDL, IDL, and LDL. The combined hormones reduced plasma concentrations of LDL-cholesterol and LDL apoB significantly by significantly increasing the FCR of LDL (Table 5). An increase in the FCR of LDL-apoB has also been observed during continuous administration of CEE + MPA (23). However, cyclical estradiol + continuous norgestrel reduce LDL pool size by inhibiting LDL production (32). Whereas a previous study involving the daily administration of 2 mg of estradiol alone to nine PMP women (23) found a significant 21% increase in LDL apoB (13) production, the present study found only a trend towards a small increase (mean 10%, Table 5) suggesting the possibility that MP and/or other progestins could modulate the effects of estrogens on LDL kinetics. Pharmacological doses of ethinyl estradiol in the rabbit have been shown to increase LDL receptor expression (43) and the increase in LDL fractional catabolism with the present hormones could be explained by estrogen-enhancement of LDL receptor activity.

Parameters of LDL metabolism in the present study were determined by multicompartmental analysis of radioiodinated LDL, which remains a reference method for determining LDL-FCR (13). The mean control value for LDL-FCR in the present PMP women of 0.269 pools/d was in the same range as that obtained using similar technology in other groups of estrogen-deficient PMP women (23, 32, 44, 45). Comparisons between the present findings involving exogenous labeling versus studies involving endogenous labeling of LDL should take into account the different experimental methods and different subject

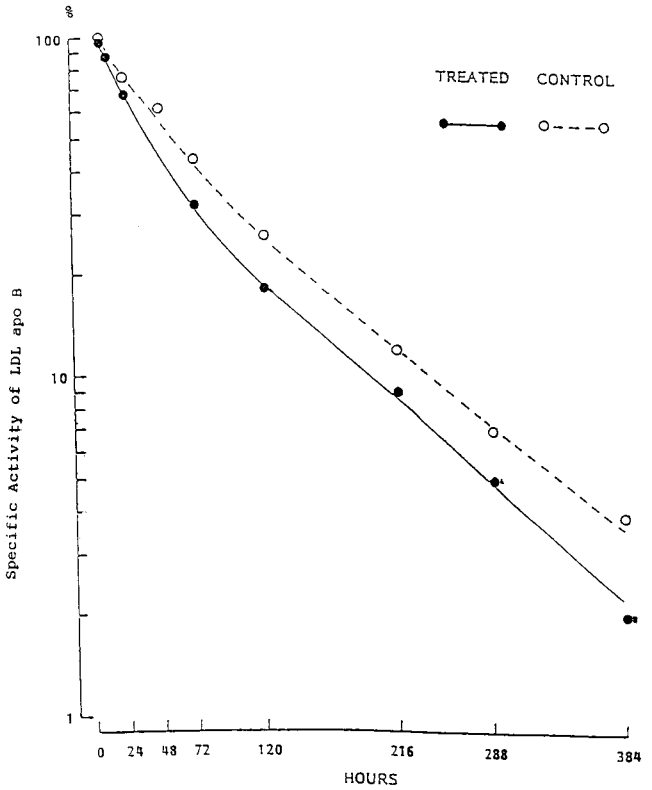

Fig. 3. Specific radioactivity of LDL apoB expressed as percent of the peak specific activity after intravenous injection of autologous ^{125}I-labeled LDL for subject no 1.

populations. Values for LDL-FCR derived from endogenous labeling studies can be overestimated if the experimental protocol is too short, resulting in insufficient information on the LDL tracer data to support the existence of an LDL exchange pool. Similarly, overestimation results if the studies rely upon LDL tracer data derived from injected VLDL. Thus, a somewhat higher mean value for LDL-FCR (i.e., 0.36, range 0.21–0.60) has been obtained during endogenous labeling of LDL apoB in healthy young men who had mean levels of LDL cholesterol which were 13% lower than the present PMP women (46). Conversely, estrogen replacement increases values for LDL-FCR in PMP women, tending to raise them into the same range as those of healthy men with normal to borderline high plasma cholesterol (47, 48). One of the potential drawbacks of the exogenous approach is that VLDL isolated for labeling and reinjection may under-represent that portion of the VLDL population that is converted to LDL via the delipidation pathway. In contrast, endogenous labeling may allow the experimentalist to see the kinetics of all VLDL particles, leading to the conclusion that a larger proportion of LDL is derived from the VLDL fraction that is seen with exogenous labeling. However, the mean control value for direct production of LDL in the present PMP woman (8.3 mg/kg/d) is similar to that previously reported in other estrogen-deficient PMP women (23, 44). The 16% reduction in the fasting plasma concentration of LDL-cholesterol (S_f 0–20) with the CEE_{cont}/MP_{cont} accords with reductions of 13–15% observed during cyclical administration of MP together with continuous or cyclical CEE (14, 24).

The present findings indicate that CEE_{cont}/MP_{cont} significantly increases IDL production (Table 4) and that this, in turn, is largely explained by increased conversion of VLDL to IDL (Table 3). However, the increase in production of IDL did not result in an increase in LDL production because of the increase in direct removal of IDL, which is consistent with up-regulation of LDL receptors by the CEE. Treatment also significantly altered the composition of IDL (S_f 12–60), as reflected in 17% lower value for the ratio of cholesterol to apoB in plasma IDL (S_f 12–60) and this explains why IDL cholesterol concentration was unchanged despite the tendency for IDL apoB pool size to increase (Tables 2 and 4).

There have been no previous reports of the effects on HDL-cholesterol of continuously adminstering both CEE and MP. However, the relatively high proportional increase in HDL-cholesterol with the present HRT could be explained, at least in part, by the observation that subjects with low HDL experience relatively larger than average increases in HDL-cholesterol during ovarian hormone replacement (49). Furthermore, it has been reported that administration of continuous CEE and cyclical MP tended to increase HDL more than CEE alone (24). However, other studies found that MP, like MPA, blunts estrogen-induced increases in HDL-cholesterol, but the reductions in increment are smaller than with MPA (14, 50).

CEE alone increases production of VLDL TG (51) and estradiol administered alone increases production of VLDL apoB (13). However, when CEE was combined with MP in the present study (Table 4) or combined with the C-19 progestin MPA (23), no significant increase in VLDL production or TG concentration was observed. This suggests that these progestins blunt the triglyceride-elevating effect of CEE, consistent with a previous report that the C-19 progestin norethindrone acetate inhibits hepatic triglyceride secretion in a swine model (50). As mentioned above, numerous short-term and some long-term studies are consistent with the view that co-administration of MPA with CEE blunts the expected estrogen-induced increments in plasma TG (15–20).

In summary, the CEE_{cont}/MP_{cont} lowered LDL cholesterol and apoB by increasing the fractional catabolism of LDL of S_f 0–12 and thereby improved the plasma lipoprotein vascular risk profile (52, 53).

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