0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 12
Printed in U.S.A.

Effect of Estrogen on Very Low Density Lipoprotein and Low Density Lipoprotein Subclass Metabolism in Postmenopausal Women*

HANNIA CAMPOS, BRIAN W. WALSH, HELENA JUDGE, AND FRANK M. SACKS

Department of Nutrition, Harvard School of Public Health (H.C., H.J., F.M.S.); the Department of Obstetrics and Gynecology, Brigham and Women's Hospital (B.W.W.); and the Channing Laboratory, Department of Medicine, Harvard Medical School (F.M.S.), Boston, Massachusetts 02115

ABSTRACT

Estrogen decreases low density lipoprotein (LDL) particle size, and smaller LDL particles are associated with coronary atherosclerosis. To understand the metabolic basis for this change, we studied the effect of oral 17β-estradiol (2 mg/day) on apolipoprotein B-100 (apoB) metabolism, in eight healthy postmenopausal women. The study was a randomized, double blinded, placebo-controlled, cross-over trial with intervention sequences of 6 weeks each. ApoB in very low density lipoprotein, intermediate density lipoprotein, and LDL subclasses was endogenously labeled with $[D_3]$L-leucine, and metabolic rates were calculated by computer modeling. The overall effect of oral estrogen therapy on apoB metabolism was to accelerate the fractional catabolic rates of all particles studied and production rates of all except IDL. For light LDL (density = 1.019–1.036 g/mL), estrogen increased the mean fractional catabolic rate by 63% from 0.59 to 0.96 pools/day ($P = 0.02$), whereas the production rate increased by a lesser amount (42%) from 575 to 817 mg/day ($P = 0.10$). These metabolic changes reduced light LDL cholesterol and apoB concentrations by 26% ($P = 0.005$) and 19% ($P = 0.03$), respectively. In contrast, dense LDL (density = 1.036–1.063 g/mL) cholesterol and apoB concentrations were unchanged by the intervention, as both the apoB fractional catabolic rate and production rate were significantly increased by similar amounts, 39% (from 0.41 to 0.57 pools/day, $P = 0.01$) and 38% (from 434 to 601 mg/day; $P = 0.003$), respectively. Estrogen decreased the predominant LDL peak particle size from 273 to 268 Å ($P = 0.04$). Thus, estrogen therapy increases the clearance of both light and dense LDL, counteracting increases in production rates. The reduced plasma residence times of light and dense LDL both may be antiatherogenic, even though, for dense LDL, the concentration did not change. (*J Clin Endocrinol Metab* **82:** 3955–3963, 1997)

ESTROGEN replacement therapy is associated with low rates of coronary heart disease (CHD) in postmenopausal women (1, 2). Some of this apparent protection with estrogen use may be attributed to reduced LDL cholesterol concentrations (3, 4). However, estrogen decreases LDL particle size (5–8), and smaller LDL particles are associated with coronary atherosclerosis (9–12). Small, dense LDL particles have atherogenic properties, such as increased binding to arterial wall proteoglycans (13), low affinity for the LDL receptor (14), and increased susceptibility to oxidation (15).

Three predominant LDL subclasses have been found in populations (16, 17). Women usually have either large, light LDL particles, termed LDL I (>267 Å; density, 1.025–1.032 g/mL), or particles with medium size and density, LDL II (255–267 Å; density, 1.032–1.038 g/mL). In men, the predominant subclasses are LDL II and the small, dense LDL III (<255 Å; density, 1.038–1.050 g/mL) (9, 18). It is well established that in men, a predominance of LDL III is associated with CHD, and this phenotype is also termed, LDL pattern B (9–12). Although women with CHD tend to have smaller LDL particles (265 Å) compared to controls (269 Å), the mean LDL particle size in women with CHD corresponds to increased LDL II rather than LDL III as found in men (12). Concern has been raised regarding the effects of estrogen on LDL size, as several studies have shown that estrogen therapy increases the relative proportions of LDL II and LDL III while decreasing the proportion of LDL I; this decreases the size of the predominant LDL particles (5–8). However, as these studies did not measure the plasma concentrations of LDL II or III, it is not known whether the relative shift to smaller LDL subclasses is caused by an increase in the plasma concentration of LDL II or III or a decrease in LDL I with estrogen use. Moreover, estrogen increases the clearance rate of unfractionated LDL (3), and it is unclear how this change in metabolism of LDL particles is linked to the changes in LDL particle size distribution. Therefore, the potential for atherogenicity from these estrogen-induced changes in LDL subclasses is unclear. For example, if the increase in the relative proportion of smaller, denser LDL particles in postmenopausal estrogen users is due to preferential removal of large LDL I particles rather than to an increase in small LDL III, it may not be regarded as unfavorable (6, 7). However, it is also possible that the mild triglyceride elevations caused by estrogen increase the concentration of small, dense LDL, because increased triglyceride concentrations is the major determinant of smaller, denser LDL (3, 17).

It is also necessary to interpret the hypertriglyceridemic effect of estrogen use, particularly as it has become estab-

Received June 24, 1997. Revision received July 22, 1997. Accepted August 11, 1997.

Address all correspondence and requests for reprints to: Hannia Campos, Ph.D., Department of Nutrition Room 353A, Building 2, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115. E-mail: hphac@gauss.bwh.harvard.edu.

* This work was supported by a grant from the National Heart, Lung, and Blood Institute (RO1-HL-34980) and from the Office for Women's Research, NIH (Bethesda, MD), and by General Clinical Research Center Grant NCRR GCRC MO1-RR-02635 to the Brigham and Women's Hospital.

lished that triglycerides are an independent risk factor for CHD in women (19, 20). Estrogen-induced hypertriglyceridemia is characterized by increased large triglyceride-rich veryl low density lipoprotein (VLDL) particles (3) similar to those found in endogenous hypertriglyceridemia (21), a lipoprotein disorder that results from impaired VLDL catabolism. VLDL particles in endogenous hypertriglyceridemia are also rich in cholesterol ester and promote cholesterol ester formation in macrophages and possibly atherosclerosis (21–24). In contrast, estrogen raises triglyceride levels by increasing the production of VLDL particles (3). Estrogen may also increase the fractional catabolic rate of VLDL, although the magnitude of this effect is smaller than the increased production rate (3). VLDL particles that are rapidly removed from the circulation are less likely to acquire cholesterol from HDL (25), are less susceptible to oxidation *in vitro* (26), and are possibly more likely to be removed by LDL receptors in the liver, all potentially antiatherogenic, rather than proatherogenic, changes. As our previous study considered a simple model for VLDL metabolism, a more refined, comprehensive approach could lead to further insights on the atherogenicity of the effects of estrogen.

We examined the effect of estrogen on the metabolism of apolipoprotein B-100 (apoB) in light and dense LDL in postmenopausal women. We also studied the metabolism of VLDL subclasses and intermediate density lipoprotein (IDL) to determine whether estrogen affects the metabolic pathways that lead to LDL formation. This study expands our previous report on the effect of estrogen on VLDL subclasses and LDL metabolism (3) by including IDL and light and dense LDL subclasses as well as a refined treatment of the intrahepatic synthetic process for apoB and of plasma VLDL kinetics. An understanding of the metabolic processes affected by estrogen may lead to inferences about the role of estrogen in protection against atherosclerosis.

Materials and Methods

Study design

The study was a randomized, double blinded, placebo-controlled, cross-over trial, previously described (3). Healthy postmenopausal women were assigned to one of three sequences of placebo, oral micronized estradiol tablets (2 mg/day; Estrace, Mead Johnson, Evansville, IN), and transdermal estradiol patches (0.1 mg twice a week; Estraderm, Ciba-Geigy, Summit, NJ) for 6 weeks each. Compliance was verified by significant reductions in plasma FSH levels of 34% during estrogen therapy (3). Participants were instructed to follow their usual diet and pattern of exercise during the study.

Subjects

Nine women were enrolled and completed the study. The metabolism of their VLDL subclasses and LDL in this trial was previously reported (3). Of these, eight had sufficient plasma samples available to measure apoB metabolism in IDL and LDL subclasses, and these women comprised the present study. The transdermal intervention period was not included in the present study because transdermal estradiol patches did not significantly alter the concentrations or metabolic rates of VLDL and LDL (3). The enrollment criteria were postmenopausal status, defined as amenorrhea for at least 10 months; a serum FSH concentration above 50 IU/L total cholesterol and triglyceride concentrations below the 95th percentile for age (27); nonsmoking; not obese (<150% of their ideal body weight); not diabetic; not taking medications known to affect lipoprotein metabolism or blood pressure; and alcoholic beverage consumption less than 28 mL ethanol daily. The subjects' characteristics were mean age of 55 ± 7 (range, 44–63 yr) and mean weight of 62.6 ± 5 (range, 54–69 kg). This study was approved by the institutional review board of the Brigham and Women's Hospital, and informed consent was obtained from all subjects.

Experimental protocol

Subjects were admitted to the Clinical Research Center, Brigham and Women's Hospital, after a 12-h overnight fast. Estrogen or placebo pills were taken approximately 9 h before admission. A nonradioactive isotope [D_3]L-leucine (purity, >99%; Tracer Technologies, Cambridge, MA) was used to endogenously label apoB-containing lipoproteins. Subjects were given an iv bolus injection (4.2 μmol/kg) of [D_3]L-leucine, immediately followed by a constant 14-h iv infusion delivered at a rate of 4.8 μmol/kg BW·h. Blood specimens were obtained from a second iv catheter in the contralateral arm every 10 min for the first 90 min and hourly thereafter. All blood specimens obtained during these infusions were processed immediately. During the first 4 h of the study, subjects consumed exclusively noncaloric, noncaffeinated fluids. This period was followed by the consumption of a standardized fat- and leucine-free diet containing 60% of the total daily calories required for maintenance, served as lunch at 4 h, dinner at 10 h, and an evening snack at 13 h. Each subject was served the exact same meals for both admissions. For determination of overnight fasting lipoprotein measurements, blood samples were collected on three occasions over a period of 2 weeks. Plasma was separated by centrifugation at 4 C and stored at −80 C for analyses.

Isolation and quantification of lipoproteins

VLDL subfractions with Svedberg units of flotation (S_f) 60–400 (light VLDL) and S_f 20–60 (dense VLDL) were prepared by cumulative rate, density-gradient ultracentrifugation using a type 41 SW rotor (Beckman Instruments, Palo Alto, CA) (27) in an L8-70M ultracentrifuge (Beckman Instruments, Palo Alto, CA) using fresh plasma. IDL and LDL subfractions were prepared by sequential ultracentrifugation from frozen plasma after the VLDL subfractions were removed. Samples were spun in the outer row of a Beckman type 25 rotor at 25,000 rpm at 10 C for 10 h to isolate IDL (density = 1.006–1.019 g/mL), for 16 h for light LDL (density, 1.019–1.035 g/mL), and for 20 h for dense LDL (density 1.035–1.063 g/mL). By definition, light LDL included predominantly LDL I and LDL II particles, and dense LDL included predominantly LDL II and LDL III particles (16). Densities were adjusted with potassium bromide, and accuracy was confirmed by measurement of refractive index of the potassium bromide solution in tubes centrifuged in parallel without plasma as well as in the lipoprotein-free clear zones beneath the floated lipoproteins.

Plasma and lipoprotein fractions were assayed for total cholesterol and triglyceride using enzymatic reagents (Boehringer Mannheim, Indianapolis, IN). Cholesterol determinations in our laboratory are standardized according to the program for research laboratories specified by the Centers for Disease Control and the NHLBI. Enzyme-linked immunosorbent assay using polyclonal antibodies and alkaline phosphatase conjugate of the same antibody were used to measure apoB in plasma and isolated fractions. The standards used for measuring apoB in our current study were calibrated to the improved standards of the International Federation of Clinical Chemistry, which yields higher values (by 1.41) than the older standards from the Centers for Disease Control that were used in the previous study (3). LDL predominant peak size (Å) and the area distribution of large and small LDL particles were determined by electrophoresis of whole plasma on nondenaturing 2–16% polyacrylamide gradient gels stained for lipid with oil red O as previously described (6). LDL particle size was estimated from calibration curves using latex beads and Pharmacia (Uppsala, Sweden) high mol wt standards for reference.

Measurement of stable isotope enrichment

ApoB was precipitated with isopropanol and washed three times (28), and the absence of apoproteins other than apoB was confirmed by SDS-PAGE. ApoB was hydrolyzed with 6 N HCl under nitrogen for 16 h at 120 C. Amino acids were converted to volatile heptafluorobutyric acid derivatives by heating with an acetyl chloride and propanol mixture at 110 C for 25 min, drying under N_2, and heating with heptafluorobutyric

acid anhydride for 25 min at 60 C. The specimens were dried under N_2 and dissolved in ethyl acetate for injection into a 5890 gas chromatograph and a 5988A mass spectrometer (Hewlett-Packard, Palo Alto, CA) as previously described (3).

Calculation of metabolic rates

The apoB metabolic parameters were calculated using the NIH CONSAM 31 multicompartmental modeling program. This program uses the rates of appearance of deuterated leucine in apoB for each lipoprotein subclass and their plasma pool sizes to calculate metabolic rates. Our previous report of these subjects used a model for the metabolism of light and dense VLDL (3) and calculated LDL metabolism from the linear slope of the LDL isotopic enrichment curve. We have since refined the model of VLDL subclasses and added IDL and two LDL subclasses. The new model was created with the data from the placebo period using these guidelines: 1) apoB follows a stepwise delipidation cascade starting from light VLDL and ending with dense LDL; 2) allow direct production from the liver for dense VLDL, IDL, and LDL (light and dense) only if required for an optimal fit; 3) conserve mass along the delipidation cascade (*i.e.* apoB will leave the plasma compartment between light VLDL and dense LDL only if required); and 4) all plasma apoB compartments are lipoprotein subclasses directly isolated. Theoretical subcompartments (plasma or extravascular) are added only if required for an optimal fit. Production rates (fluxes) were calculated as the products of fractional catabolic rates and pool size. Pool size (milligrams) was calculated by multiplying body wt (kilograms) by 0.44 times the plasma concentration (milligrams per dL). All subjects were first modeled independently by three investigators (H.C., B.W.W., and F.M.S.). Consensus was reached for the best-fit curve for each compartment and for the use of alternative pathways or compartments by comparing the residual sum of squares.

Light VLDL was the first lipoprotein to be labeled and had a delay of approximately 35 min for intrahepatic synthesis of apoB, and for assembly and secretion of VLDL (Fig. 1). After the delay, the enrichment of light VLDL increased sharply. A minimum of eight intrahepatic apoB compartments was needed to produce a sharp resolution of the intrahepatic delay to fit the steep initial rise of label in light VLDL. The enrichment curve for light VLDL apoB required the existence of two subcompartments for particles with slow and fast catabolism. The fast, light VLDL compartment was divided into four pathways: removal from circulation, conversion to slow, light VLDL, conversion to dense VLDL, and direct conversion to IDL (bypassing dense VLDL; Fig. 2). Dense VLDL was either removed from the circulation or converted to IDL. IDL was either removed or converted to light LDL or dense LDL (bypassing light LDL). Light LDL was either removed or converted to dense LDL. Extravascular compartments were required between light VLDL and IDL and between IDL and dense LDL. In sum, the final model has a lipolytic cascade from light VLDL to dense LDL, and direct production and removal of particles in all density ranges. A diagram of the model structure is shown in Fig. 2.

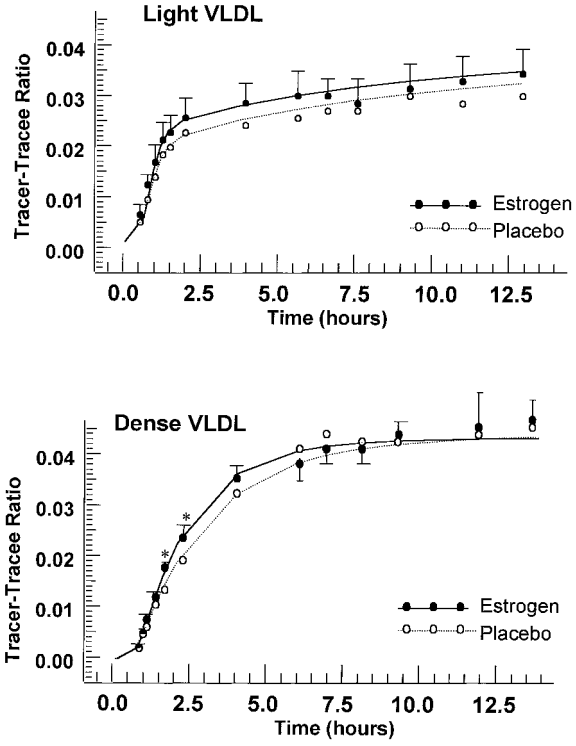

FIG. 1. Effect of estrogen on the isotopic enrichment of leucine in apoB of light (*top*) and dense (*bottom*) VLDL. *Bars* represent the standard error of the intra-individual differences between the treatments; n = 8; *$P \leq 0.05$ for paired differences for individual two points.

Statistical analysis

All data were analyzed with the Statistical Analysis System software (SAS, Cary, NC). The treatment effect was defined as the difference in plasma concentrations or metabolic rates measured during the placebo and estrogen treatments. The differences were tested using two-tailed paired *t* tests.

Results

Plasma lipoprotein concentrations

Estrogen therapy significantly increased light VLDL apoB and triglyceride concentrations by 25% and 24%, respectively ($P \leq 0.05$; Table 1). There were no significant changes in dense VLDL and IDL concentrations. Estrogen reduced light LDL cholesterol (−26%; $P = 0.005$) and apoB (−19%; $P = 0.02$) concentrations as well as the cholesterol to apoB ratio (−12%; from 0.038 ± 0.004 to 0.033 ± 0.005; $P = 0.004$). Estrogen did not affect these parameters in dense LDL. Measurements of LDL size by gradient gel electrophoresis demonstrated a significant decrease in the proportion of LDL I particles, an increase in the proportion of LDL II particles ($P \leq 0.05$), and no change in LDL III particles. The predominant LDL peak particle size was significantly reduced from 273 to 268 Å ($P \leq 0.05$). With placebo, the major LDL subclass was LDL I, whereas with estrogen, it was LDL II. None of the subjects receiving either treatment was classified as having predominantly LDL III. The smallest predominant LDL size detected was 265 Å. Therefore, the LDL subclass measurements of density and size together indicate that the shift in distribution in LDL size from LDL I to LDL II was due to a decrease in particle concentration of large, light LDL rather than an increase in small, dense LDL. The relative decrease in LDL I could be due to a decrease in both particle number and cholesterol content.

VLDL metabolism

Estrogen increased the production rate of light VLDL by 64% ($P = 0.08$; Table 2). Estrogen exclusively affected the production rate of the fast compartment of light VLDL particles, representing 76–78% of light VLDL particle mass; the production rate of slow, light VLDL particles was unchanged. This additional light VLDL flux induced by estrogen was removed from the circulation before conversion to dense VLDL or IDL, as shown in Table 3 and Fig. 2. Estrogen also significantly increased the conversion of light VLDL to dense VLDL by 2.2-fold ($P = 0.02$) while reducing the direct

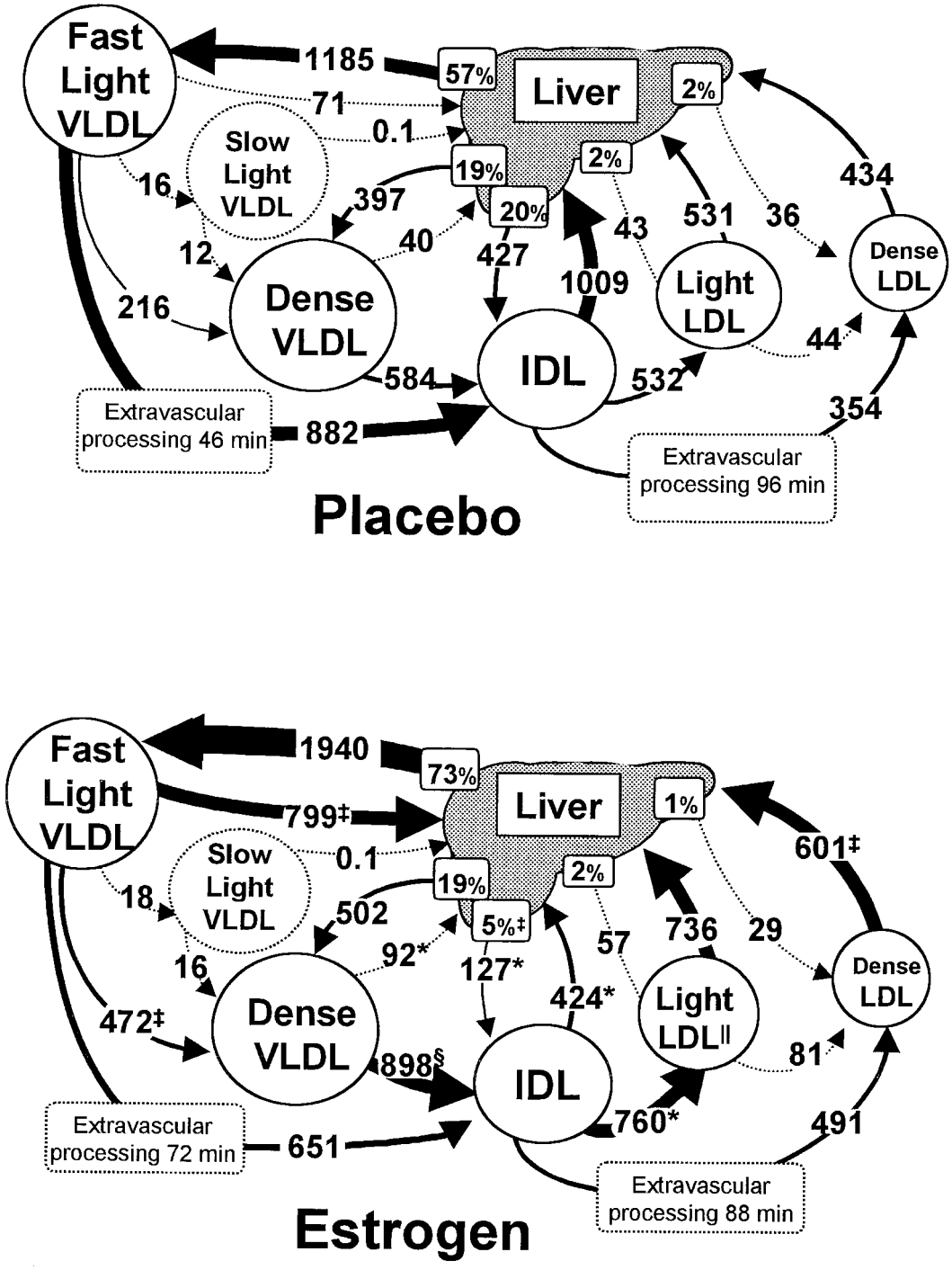

FIG. 2. Model of plasma apoB metabolism in postmenopausal women; placebo (*top*) and estrogen (*bottom*) treatments. The percentages shown inside the liver indicate the proportion of apoB directly secreted into each compartment. The width of the *arrows* illustrates the quantity of lipoprotein passing through each metabolic pathway (flux), expressed in mg of apoB per day. The *arrows* that point away from the liver represent direct secretion of particles. The *arrows* pointing towards the liver indicate clearance from plasma. The plasma lipoprotein subclasses sampled and directly measured are shown inside the *circles with solid lines*. Theoretical lipoprotein compartments (not sampled or directly measured) are depicted with *dotted lines*. These are a light VLDL plasma compartment for particles with slow clearance, and compartments for extravascular processing of fast light VLDL to IDL, and of IDL to dense LDL. The predominant pathway for flux is production of fast, light VLDL, lipolysis, and extravascular processing to IDL bypassing dense VLDL, and clearance of IDL. Compared with the results for placebo treatment, predominant fluxes changed with estrogen (*bottom*). Production of apoB containing particles is shifted from denser to lighter particles, a major cycle of flux emerges for fast light VLDL with increased hepatic production and substantial removal, increased flux of IDL arising from dense VLDL rather than from hepatic production, and redirection of IDL flux from direct clearance to conversion to LDL subclasses. Note however that estrogen treatment enhanced fractional catabolic rates of both LDL subclasses, resulted in decreased light LDL pools size, and no change in dense LDL pools size (Table 2). *$P \leq 0.10$; ‡$P \leq 0.05$; §$P \leq 0.01$ indicate the significance levels of flux changes on estrogen compared with placebo. ‖$P \leq 0.05$, significant level of pool size change.

conversion of light VLDL to IDL via the extravascular processing compartment. Overall flux of light VLDL undergoing lipolysis to smaller particles was unchanged (1114 mg/day placebo *vs.* 1141 mg/day estrogen), as increases in the conversion of light VLDL to dense VLDL were balanced by concomitant decreases in direct conversion to IDL. Estrogen did not significantly affect the fractional catabolic rates of the fast or slow compartments of light VLDL, as demonstrated by the similarity of slopes in the light VLDL enrichment curves during both treatments (Fig. 1, *top panel*).

Marked differences between placebo and estrogen were found in the metabolism of dense VLDL (Table 2). Estrogen increased the dense VLDL fractional catabolic rate by 45% ($P = 0.008$), which is illustrated by a greater slope for the dense VLDL enrichment curve during estrogen therapy than during placebo administration (Fig. 1, *bottom panel*). The dense VLDL production rate was increased by 59% ($P = 0.01$) with estrogen compared to placebo. The increased production of dense VLDL (percentage of the total flux) originated mainly from light VLDL lipolysis rather than from direct liver secretion (Table 3 and Fig. 2). As both dense VLDL production and catabolism were increased by estrogen to a similar extent, the dense VLDL concentration was not significantly changed. The increased production of dense VLDL particles resulted in significantly increased flux of particles to IDL (+54%; $P = 0.008$). Like the effects of estrogen on light VLDL, direct removal of dense VLDL increased, but this pathway was relatively unimportant and contained only 4% of the dense VLDL flux compared to 31% of the light VLDL flux in estrogen.

TABLE 1. Effect of oral estrogen on plasma lipoprotein subclass composition and LDL particle size in postmenopausal women

Parameter	Placebo	Estrogen
Plasma		
Cholesterol (mmol/L)	4.73 ± 0.78	4.40 ± 0.67[a]
Triglyceride (mmol/L)	0.76 ± 0.23	0.85 ± 0.32
ApoB (mg/L)	92 ± 20	83 ± 21[b]
Light VLDL		
Cholesterol (mmol/L)	0.11 ± 0.05	0.11 ± 0.06
Triglyceride (mmol/L)	0.17 ± 0.08	0.21 ± 0.12[a]
ApoB (mg/L)	1.6 ± 1.0	2.0 ± 1.3[a]
Dense VLDL		
Cholesterol (mmol/L)	0.12 ± 0.07	0.11 ± 0.08
Triglyceride (mmol/L)	0.11 ± 0.07	0.12 ± 0.08
ApoB (mg/L)	2.3 ± 1.7	2.7 ± 2.0
IDL		
Cholesterol (mmol/L)	0.15 ± 0.06	0.13 ± 0.08
Triglyceride (mmol/L)	0.06 ± 0.02	0.07 ± 0.04
ApoB (mg/L)	7.9 ± 5.4	7.8 ± 7.5
Light LDL		
Cholesterol (mmol/L)	1.38 ± 0.37	1.02 ± 0.35[b]
Triglyceride (mmol/L)	0.09 ± 0.03	0.09 ± 0.04
ApoB (mg/L)	37 ± 7	30 ± 7[a]
Dense LDL		
Cholesterol (mmol/L)	1.35 ± 0.34	1.28 ± 0.31
Triglyceride (mmol/L)	0.10 ± 0.04	0.10 ± 0.03
ApoB (mg/L)	39 ± 8	38 ± 8
LDL particle size		
Predominant peak (Å)	273 ± 5	268 ± 2[a]
Large LDL I (% area)	57 ± 25	27 ± 13[a]
Medium LDL II (% area)	36 ± 24	65 ± 12[a]
Small LDL III (% area)	7 ± 5	8 ± 6

Values are given as the mean ± SD (n = 8). Recovery of apoB, based on the sum of all fractions was 96%.
[a] $P \leq 0.05$.
[b] $P \leq 0.01$.

IDL metabolism

In contrast to VLDL, estrogen did not significantly affect either the IDL production rate (1894 *vs.* 1675 mg/day; $P = 0.4$) or the fractional catabolic rate (9 *vs.* 11 pools/day; $P = 0.1$; Table 2 and Fig. 3). However, the origin and fate of IDL were significantly altered by estrogen therapy (Table 3 and Fig. 2). Compared to placebo, the proportion of total IDL production that originated from direct liver secretion was decreased by estrogen from 23% to 8% ($P = 0.06$). Concomitantly, IDL that arose from lipolysis of dense VLDL was increased from 31% to 52% ($P = 0.008$). Direct production of IDL from light VLDL was similar with both placebo and estrogen (45% *vs.* 40%). IDL flux was significantly shifted by estrogen from direct removal from the circulation to conversion to light and dense LDL. Thus, although estrogen did

TABLE 2. Effect of oral estrogen on VLDL, IDL, and LDL apoB metabolic rates, and pool sizes in postmenopausal women

Lipoprotein subclass	Fractional catabolic rate (pools/day)		Production rate (flux) (mg/day)		Pool size (mg)	
	Placebo	Estrogen	Placebo	Estrogen	Placebo	Estrogen
Light VLDL	32 ± 13 (9–46)	38 ± 13 (27–67)	1185 ± 596 (414–2423)	1940 ± 1061[a] (636–3644)	45 ± 26 (10–88)	54 ± 33[a] (15–104)
Fast light VLDL	43 ± 18 (11–68)	53 ± 20 (35–95)	1185 ± 596 (414–2423	1940 ± 1061[a] (636–3644)	34 ± 20 (6–69)	42 ± 29 (9–94)
Slow light VLDL	1.4 ± 0.9 (0.5–3.1)	1.4 ± 0.9 (0.2–2.6)	12 ± 8 (4–29)	16 ± 13 (2–45)	11 ± 9 (4–31)	12 ± 6 (6–21)
Dense VLDL	11 ± 3 (6–17)	16 ± 6[b] (6–27)	624 ± 386 (130–1332)	990 ± 646[b] (469–2213)	61 ± 41 (10–120)	70 ± 47 (27–154)
IDL	9 ± 3 (5–12)	11 ± 5 (3–19)	1894 ± 600 (1187–3050)	1675 ± 374 (1216–2410)	238 ± 124 (155–522)	210 ± 183 (582–622)
Light LDL	0.59 ± 0.21 (0.22–0.86)	0.96 ± 0.29[c] (0.68–1.62)	575 ± 163 (235–732)	817 ± 338[a] (515–1581)	1013 ± 182 (704–1244)	846 ± 180[c] (550–1058)
Dense LDL	0.41 ± 0.16 (0.22–0.59)	0.57 ± 0.21[b] (0.24–0.86)	434 ± 176 (122–655)	601 ± 238[c] (313–1015)	1087 ± 174 (847–1307)	1084 ± 208 (671–1331)

Values are given as the mean ± SD. Minimum and maximum values for each parameter are given in *parentheses*.
[a] $P \leq 0.10$.
[b] $P \leq 0.01$.
[c] $P \leq 0.05$.

TABLE 3. Effect of oral estrogen on VLDL, IDL, and LDL apoB flux in postmenopausal

	Placebo		Estrogen	
	Flux (mg/day)	% of total[a]	Flux (mg/day)	% of total[a]
Fast, light VLDL[b]				
Removed before conversion	71 ± 200	5 ± 13	799 ± 764[c]	31 ± 26[c]
Converted to slow, light VLDL	16 ± 16	1 ± 1	18 ± 13	1 ± 1
Converted to dense VLDL	216 ± 372	17 ± 30	472 ± 595[c]	23 ± 25
Converted to IDL (directly)	882 ± 432	77 ± 30	651 ± 365	45 ± 31[c]
Slow, light VLDL				
Removed before conversion	0.1 ± 0.2	0.6 ± 0.8	0.1 ± 0.1	0.1 ± 0.3
Converted to dense VLDL	12 ± 8	99 ± 0.9	16 ± 14	100 ± 0.3
Dense VLDL				
From direct production	397 ± 158	78 ± 30	502 ± 116	63 ± 29[c]
From slow, light VLDL	12 ± 8	2 ± 1	16 ± 14	2 ± 1
From fast, light VLDL	216 ± 372	20 ± 31	472 ± 595	33 ± 29[c]
Removed before conversion	40 ± 113	7 ± 20	92 ± 261[d]	4 ± 12
Converted to IDL	584 ± 408	93 ± 20	898 ± 477[e]	96 ± 12
IDL				
From direct production	427 ± 430	23 ± 22	127 ± 164	8 ± 11[c]
From fast, light VLDL	882 ± 432	45 ± 19	651 ± 365	40 ± 21
From dense VLDL	584 ± 408	31 ± 22	898 ± 477[e]	52 ± 20[e]
Removed before conversion	1009 ± 751	49 ± 21	424 ± 474[d]	22 ± 20[c]
Converted to light LDL	532 ± 205	32 ± 17	760 ± 261[d]	47 ± 17[d]
Converted to dense LDL	354 ± 157	19 ± 8	491 ± 292	31 ± 17[c]
Light LDL				
From direct production	44 ± 69	11 ± 20	57 ± 88	5 ± 6
From IDL	532 ± 205	89 ± 20	760 ± 474[d]	95 ± 6
Removed before conversion	531 ± 231	85 ± 34	735 ± 369	90 ± 19
Converted to dense LDL	44 ± 79	15 ± 34	81 ± 142	10 ± 19
Dense LDL[a, b]				
From direct production	36 ± 64	9 ± 14	29 ± 53	8 ± 14
From light LDL	44 ± 79	8 ± 13	81 ± 142	16 ± 27
From IDL	354 ± 157	83 ± 13	491 ± 292	77 ± 33

Values are given as the mean ± SD (n = 8).

[a] The proportion of total flux for each pathway in each lipoprotein subclass. As these percentages were the means of the percentages for the individual subjects, the results are not equivalent to the percentages calculated from the mean fluxes.

[b] All light VLDL originated in the liver, and all dense LDL was removed from the circulation.

[c] $P \leq 0.05$ *vs.* placebo.

[d] $P \leq 0.1$ *vs.* placebo.

[e] $P \leq 0.01$ *vs.* placebo.

not change the overall IDL production or fractional catabolic rates, it directed IDL flux from clearance to lipolysis, thereby increasing light and dense LDL flux.

LDL metabolism

During placebo or estrogen treatment, the fractional catabolic rates for light LDL were higher than those for dense LDL ($P = 0.07$ for placebo; $P = 0.04$ for estrogen; Table 2). Estrogen significantly increased the fractional catabolic rate of light LDL by 63% ($P = 0.02$) and that of dense LDL by 39% ($P = 0.003$; Table 2 and Fig. 4). Interestingly, estrogen also increased production rates of both light LDL (+42%; $P = 0.10$) and dense LDL (+38%; $P = 0.03$). Nearly all of the increases in light and dense LDL production rate came from IDL lipolysis (+228 for light and +137 mg/day for dense LDL) and not from direct liver production. Less than 12% of LDL flux, light or dense, came from direct liver production. Production of dense LDL from light LDL was also low (8% with placebo and 16% with estrogen). Although total flux of light LDL increased, its increased fractional catabolic rate overcame the increased production rate, and the net effect was a significant reduction in light LDL concentrations with estrogen compared to placebo. In contrast, the concentration of dense LDL did not change because the increases in production and fractional catabolic rates exactly compensated for each another.

Intrahepatic metabolism and extravascular processing

No significant estrogen effect was detected between the time elapsed from the start of the [D_3]L-leucine infusion and the appearance of label in plasma light VLDL apoB (delay = 35 *vs.* 37 min). Estrogen increased the total apoB liver production by 27%, from 2089 to 2655 mg/day, but this difference was not statistically significant. However, estrogen significantly altered the distribution of particles newly synthesized by the liver. Light VLDL comprised 57% of total apoB production with placebo and 73% with estrogen ($P = 0.06$), whereas IDL decreased from 20% to 5% ($P = 0.05$). Two compartments for extravascular processing, one between light VLDL and IDL and one between IDL and dense LDL, were required by the data in both treatments. Estrogen did not affect the residence times in these compartments (Fig. 2).

Discussion

The overall effect of oral estrogen therapy on apoB metabolism was to accelerate the fractional catabolic rates of all

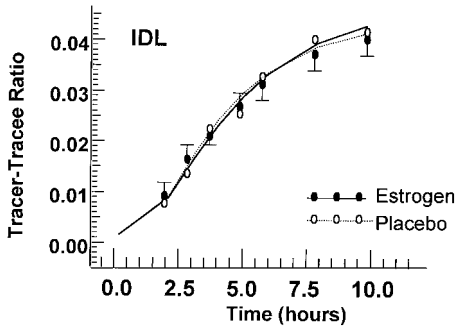

FIG. 3. Effect of estrogen on the isotopic enrichment of leucine in apoB of IDL. See Fig. 1 for legend.

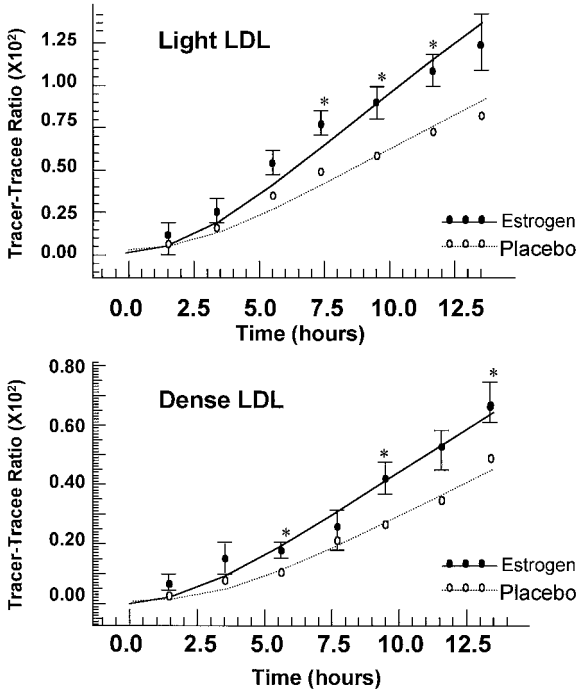

FIG. 4. Effect of estrogen on the isotopic enrichment of leucine in apoB of light (*top*) and dense (*bottom*) LDL.

particles studied and the production rates of all except IDL. These metabolic alterations raised the plasma concentration of light VLDL particles and light VLDL triglyceride as well as lowered the concentration of light LDL particles and light LDL cholesterol. These changes in lipoprotein lipid concentrations explain the increased concentrations of plasma total triglyceride and decreased concentrations of cholesterol that are caused by oral estrogen therapy.

The greatest effect of estrogen on LDL metabolism was to increase the fractional catabolic rate of light LDL particles by 63%. This caused a significant reduction in the plasma concentration of light LDL particles, overcoming the 42% increase in production rate. Consistent with previous studies of estrogen therapy (5–8), we found that the relative proportion of smaller LDL particles, as measured by gradient gel electrophoresis, was increased, and the proportion of larger particles was decreased. This shift in relative distribution of LDL particles caused a decrease in the predominant LDL peak particle size. However, our data clearly show that the relative increase in smaller LDL particles in women taking estrogen is due to preferential lowering of the concentration of light LDL I particles and not to increased concentration of denser LDL particles. Despite no effect on the dense LDL concentration, estrogen had significant effects on its metabolism, increasing the fractional catabolic rate by 39% and the production rate by 38%.

Increased light and dense LDL catabolism in women receiving estrogen therapy is probably due to up-regulation of LDL receptors (29, 30) and possibly to increased clearance by LDL receptor-independent pathways (31). Estrogen up-regulates LDL receptor activity in animal models (29) and humans (30) by increasing hepatic LDL receptor messenger ribonucleic acid transcription (32). Estrogen increases LDL receptor-independent catabolism of apoB in hypercholesterolemic rabbits (31). Up-regulation of LDL receptors, as a mechanism for LDL lowering, may be why estrogen enhanced light LDL clearance more than dense LDL clearance, although the differences in clearance rates between the subclasses were not significant. The lower fractional catabolic rates for denser compared to lighter LDL particles in our study could be attributed to their decreased binding affinity to receptors (14), perhaps resulting from reduced apoB epitope exposure (33, 34). The lower affinity of dense LDL could explain why up-regulation of LDL receptors by estrogen has less effect on clearance of dense than light LDL.

These effects of estrogen on both LDL subclasses may be beneficial, as the plasma residence times were reduced from 1.7 to 1.0 days in light LDL and from 2.4 to 1.8 days in dense LDL. Smaller, denser LDL particles are considered potentially atherogenic because of low affinity for fibroblast LDL receptor (14) and increased susceptibility to oxidation (15). With a reduced residence time, the likelihood is diminished that a dense LDL particle will enter the arterial wall rather than be cleared by LDL receptors on liver and steroidogenic tissue. The reduced *in vivo* residence time of LDL was correlated with decreased *in vitro* susceptibility to oxidation (26). Large, light LDL particles may also be atherogenic. Cholesterol-rich, large, light LDL particles cause atherosclerosis in monkeys fed a high fat diet (35) and have been associated with CHD in normolipidemic men (36). It is likely that estrogen reduced the concentration of the largest cholesterol ester-rich LDL I particles, as we found a significant reduction in the cholesterol to apoB ratio of light LDL.

Estrogen increased light VLDL production, which was responsible for increasing the light VLDL particle concentration; the mechanism is probably increased hepatocyte apoB messenger ribonucleic acid transcription and incorporation into nacent VLDL (32). The increment in light VLDL flux caused by estrogen therapy was nearly identical to the increment in the direct removal of light VLDL particles. This suggests that the newly synthesized light VLDL particles were removed from the circulation by a very rapid high affinity uptake process without undergoing lipolysis to more dense apoB-containing lipoproteins. There was also a shift in the lipolysis pathway for light VLDL, whereby conversion to IDL in an extravascular sequestration site was reduced, and conversion to dense VLDL was increased. Although this change increased the production of dense VLDL, the dense VLDL concentration remained the same, as the increased

production was balanced by an increased fractional catabolic rate. An increased VLDL concentration is a distinct characteristic in endogenous hypertriglyceridemia (21). However, the metabolism of VLDL particles in women taking estrogen is very different from that of hypertriglyceridemic patients (21). In the latter, catabolism of VLDL is impaired, whereas catabolism was increased with estrogen use. VLDL particles that are rapidly removed from the circulation are less likely to acquire atherogenic properties such as cholesterol ester enrichment (25), undergo oxidation (26), or be taken up by slow pathways such as the arterial wall (21–24). Therefore, it is unlikely that an increase in VLDL particles that have reduced residence times will promote atherosclerosis.

Estrogen did not affect the concentration or metabolism of slow light VLDL particles, which represented 22% of the light VLDL mass. The fractional catabolic rates of these VLDL particles (1.4 pools/day) were slower than those of IDL (9–11 pools/day) and approached the range for light LDL (0.59–0.96 pools/day). We hypothesize that the slow, light VLDL compartment is comprised of particles with increased apoCIII content. ApoCIII inhibits VLDL binding to the LDL receptor and to the LDL receptor-related protein (37) and inhibits triglyceride hydrolysis by both lipoprotein and hepatic lipase (38). Because of these potentially atherogenic characteristics, it is important to know that the mechanism by which estrogen increases triglyceride levels does not involve such triglyceride-rich particles with slow catabolic rates.

The changes in IDL metabolism due to estrogen use demonstrate a central function for IDL. IDL serves as a focal point for the direction of apoB flux, determining major precursor-product relationships. Estrogen significantly altered synthetic and catabolic pathways for IDL, without changing total IDL flux or the overall fractional catabolic rate. Estrogen decreased direct liver secretion of IDL, but this was balanced by increased secretion of VLDL and increased conversion of dense VLDL to IDL. This is consistent with the known effects of estrogen to increase hepatic triglyceride synthesis, which would increase the size of the apoB-containing particles that are secreted (39, 40). Estrogen also altered the clearance pathway of IDL, decreasing removal of IDL from the circulation while increasing its conversion to light and dense LDL, thereby accounting for the increased LDL production. Overall, the predominant pathway of apoB flux with placebo treatment is from fast, light VLDL directly to IDL, then splitting equally into clearance from the circulation and conversion to LDL. In contrast, with estrogen treatment, lipolytic flux from larger to smaller particles is enhanced, beginning with light VLDL, progressing to dense VLDL and IDL, and ending with light and dense LDL.

The effect of estrogen on VLDL and LDL metabolism, reported in this study, is in agreement with our previous report (3). That is, estrogen therapy increased VLDL and LDL flux, direct removal of light VLDL from the circulation, and the VLDL and LDL fractional catabolic rates. However, the metabolic rates and apoB concentrations in the present study differ from those in the previous report (3). The current model for VLDL subclasses now includes eight intrahepatic pools for the synthesis and secretion of VLDL, which take approximately 35 min. The light VLDL enrichment curve resolves sharply and produces a better fit for the initial part of the curve. The current model also included a theoretical subcompartment for light VLDL particles with slow catabolism, further improving the fit for the enrichment curve as it approached a plateau. These changes in the apoB calibration and in the model resulted in higher fractional catabolic rates and production rates for light VLDL compared to those in our previous report. The LDL fractional catabolic rates in this study were also higher than those in our previous report, as the best fit required IDL rather than VLDL or the liver to be the major source of light and dense LDL. Higher LDL fractional catabolic rates are obtained when the apoB IDL enrichment is used as the LDL precursor rather than the apoB VLDL plateau enrichment, as IDL enrichment is well below plateau when LDL enrichment begins to appear, and fractional catabolic rates are inversely proportional to the precursor enrichment. The LDL fractional catabolic rates in women taking placebo in our current study were 0.59 and 0.41 pools/day for light and dense LDL, respectively. These values were similar to that previously reported using reinfusion of radioiodinated lipoproteins in premenopausal women (0.57 pools/day) (41) and that using deuterated leucine in men (0.48 pools/day) (42).

We propose that the diminished residence times of light and dense VLDL and those of light and dense LDL could minimize the atherogenic effects of these lipoprotein particles despite changes in the lipoprotein profile that could be interpreted as adverse (an increase in light VLDL concentration and a shift in LDL distribution to smaller and denser particles) or neutral (no change in dense VLDL or dense LDL concentrations). Plasma steady state concentrations or distributions, as the sole information on lipoproteins, could lead to misinterpretation, as the underlying metabolic state of the particles is unknown. The effects of estrogen demonstrate that increased lipoprotein production is not necessarily atherogenic. The direction of flux (out of the circulation *vs.* conversion to smaller lipoproteins) and the rate of clearance are likely to contain the metabolic information that is critical to understanding the effects on atherosclerosis. This study provides further suggestive evidence that estrogen use could protect women against atherosclerosis by improving lipoprotein transport at many points along the apoB pathway.

Acknowledgments

We thank the dedicated women who participated in this study. We are grateful to the late Loren Zech, Ph.D., for many helpful discussions and suggestions about kinetic modeling. Estradiol and placebo were donated by Mead Johnson Laboratories, Bristol Myers Squibb Co. (Evanston, IL).

References

1. **Psaty BM, Heckbert SR, Atkins D, et al.** 1993 A review of the association of estrogens and progestins with cardiovascular disease in postmenopausal women. Arch Intern Med. 153:1421–1427.
2. **Grodstein F, Stampfer MJ, Manson JE, et al.** 1996 Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. N Engl J Med. 335:453–461.
3. **Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM.** 1991 Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med. 325:1196–1204.
4. **The Writing Group for the PEPI Trial.** 1995 Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women.

The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trail. JAMA. 273:199–208.
5. **Granfone A, Campos H, McNamara JR, et al.** 1992 Effects of estrogen replacement on plasma lipoproteins and apolipoproteins in postmenopausal, dyslipidemic women. Metabolism. 41:1193–1198.
6. **Campos H, Sacks FM, Walsh BW, Schiff I, O'Hanesian MA, Krauss RM.** 1993 Differential effects of estrogen on low-density lipoprotein subclasses in healthy postmenopausal women. Metabolism. 42:1153–1158.
7. **Griffin B, Farish E, Walsh D, et al.** 1993 Response of plasma low density lipoprotein subfractions to oestrogen replacement therapy following surgical menopause. Clin Endocrinol (Oxf). 39:463–468.
8. **van der Mooren MJ, de Graff J, Demacker PN, de Haan AF.** 1994 Changes in the low-density lipoprotein profile during 17 beta-estradiol-dydrogesterone therapy in postmenopausal women. Metabolism. 43:799–802.
9. **Campos H, Genest J, Blijlevens E, et al.** 1992 Low density lipoprotein particle size and coronary artery disease. Arteriosclerosis. 12:187–195.
10. **Coresh J, Kwiterovich Jr PO, Smith HH, Bachorik PS.** 1993 Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. J Lipid Res. 34:1687–1697.
11. **Stampfer MJ, Krauss RM, Ma J, et al.** 1996 A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA. 276:882–888.
12. **Gardner CD, Fortmann SP, Krauss RM.** 1996 Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA. 276:875–881.
13. **Camejo G, Hurt-Camejo E, Bondjers G.** 1990 Effect of proteoglycans on lipoprotein-cell interactions: possible contributions to atherogenesis. Curr Opin Lipidol. 1:431–436.
14. **Campos H, Arnold KS, Balestra ME, Innerarity TL, Krauss RM.** 1996 Differences in receptor binding of low density lipoprotein subfractions. Arterioscler Thromb Vasc Biol. 16:769–801.
15. **de Graaf J, Hak-Lemmers HLM, Hectors MPC, Demacker PNM, Hendriks JCM, Stalenhoef AFH.** 1991 Enhanced susceptibility to *in vitro* oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arteriosclerosis. 11:298–306.
16. **Campos H, Blijlevens E, McNamara JR, et al.** 1992 Low density lipoprotein particle size distribution: results from the Framingham Offspring Study. Arterioscler. Thromb. 12:1410–1419.
17. **Krauss RM.** 1994 Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. Curr Opin Lipidol. 5:339–349.
18. **Krauss RM, Blanche PJ.** 1992 Detection and quantitation of LDL subfractions. Curr Opin Lipidol. 3:377–383.
19. **Lapidus L, Bengtsoon C, Lindquist O, Sigurdsson JA, Rybo E.** 1985 Triglycerides–main lipid risk factor for cardiovascular disease in women? Acta Med Scand. 217:481–489.
20. **Criqui MH, Heiss G, Cohn R, et al.** 1993 Plasma triglyceride and mortality from coronary heart disease. N Engl J Med. 328:1220–1225.
21. **Packard CJ, Munro A, Lorimer AR, Gotto AM, Shepard J.** 1984 Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. J Clin Invest. 74:2178–2192.
22. **Tatami R, Mabuchi H, Ueda K, et al.** 1981 Intermediate-density lipoprotein and cholesterol-rich very low density lipoprotein in angiographically determined coronary artery disease. Circulation. 64:1174–1184.
23. **Bersot TP, Innerarity TL, Mahley RW, Havel RJ.** 1983 Cholesteryl ester accumulation in mouse peritoneal macrophages induced by beta-migrating very low density lipoproteins from patients with atypical dysbetalipoproteinemia. J Clin Invest. 72:1024–1033.
24. **Sacks FM, Breslow JL.** 1987 Very low density lipoproteins stumulate cholesteryl ester formation in U937 macrophages. Heterogeneity and biologic variation among normal humans. Arteriosclerosis. 7:35–46.
25. **Tall A, Granot E, Brocia R, et al.** 1987 Accelerated transfer of cholesteryl esters in dyslipidemic plasma: role of cholesteryl ester transfer protein. J Clin Invest. 79:1217–1225.
26. **Walzem RL, Watkins S, Frankel EN, Hansen RJ, German JB.** 1995 Older plasma lipoproteins are more susceptible to oxidation: a linking mechanism for the lipid and oxidation theories of atherosclerotic cardiovascular disease. Proc Natl Acad Sci USA. 92:7460–7464.
27. **Lipid Metabolism Branch, Division of Heart, and Vascular Diseases, National Heart, Lung, and Blood Institute.** 1980 The Lipid Research Clinics population studies data book. Bethesda: Lipid Metabolism Branch, Division of Heart and Vascular Diseases, National Heart, Lung, and Blood Institute, NIH; publication 80–1527.
28. **Egusa G, Brady DW, Grundy SM, Howard BV.** 1983 Isopropanol precipitation method for metabolic studies of very low density lipoprotein apolipoprotein B. J Lipid Res. 24:1261–1267.
29. **Windler EET, Kovanen PT, Chao YS, Brown MS, Havel RJ, Goldstein JL.** 1980 The estradiol-stimulated lipoprotein receptor of rat liver. J Biol Chem. 255:10464–10471.
30. **Eriksson M, Berglund L, Rudling M, Henriksson P, Angelin B.** 1989 Effects of estrogen on low density lipoprotein metabolism in males. Short-term and long-term studies during hormonal treatment of prostatic carcinoma. J Clin Invest. 84:802–810.
31. **Colvin Jr PLJ.** 1996 Estrogen increases low-density lipoprotein receptor-independent catabolism of apolipoprotein B in hyperlipidemic rabbits. Metabolism. 45:889–896.
32. **Srivastava RAK, Baumann D, Schonfeld G.** 1993 *In vivo* regulation of low-density lipoprotein receptors by estrogen differs at the post-transcriptional level in rat and mouse. Eur J Biochem. 216:527–538.
33. **Teng B, Sniderman A, Kauss RM, Kwiterovich ROJ, Milne RW, Marcel IL.** 1985 Modulation of apolipoprotein B antigenic determinants in human low density lipoprotein subclasses. J Biol Chem. 260:5067–5072.
34. **McNamara JR, Small DM, Li Z, Schaefer EJ.** 1996 Differences in LDL subspecies involve alterations in lipid composition and conformation changes in apolipoprotein B. J Lipid Res. 37:1924–1935.
35. **Rudel LL, Parks JS, Johnson FL, Babiak J.** 1986 Low density lipoproteins in atherosclerosis. J Lipid Res. 27:465–474.
36. **Campos H, Roederer GO, Lussier-Cacan S, Davignon J, Krauss RM.** 1995 Predominance of large LDL and reduced HDL_2 cholesterol in normolipidemic with coronary artery disease. Arterioscler Thromb Vasc Biol. 15:1043–1048.
37. **Kowal RC, Herz J, Weisgraber KH, Mahley RW, Brown MS, Goldstein JL.** 1990 Opposing effects of apolipoproteins E and C on lipoprotein binding to low density lipoprotein receptor-related protein. J Biol Chem. 265:10771–10779.
38. **Ginsberg HN, Le NA, Goldberg IJ, et al.** 1986 Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase *in vivo*. J Clin Invest. 78:1287–1295.
39. **Glueck CJ, Fallat RW, Scheel D.** 1975 Effects of estrogenic compounds on triglyceride kinetics. Metabolism. 24:537–545.
40. **Kissebah AH.** 1973 Mechanism of hypertriglyceridaemia associated with contraceptive steroids. Horm Res. 5:184–190.
41. **Schaefer EJ, Foster DM, Zech LA, Lindgren FT, Brewer HBJ, Levy RI.** 1983 The effects of estrogen administration on plasma lipoprotein metabolism in premenopausal females. J Clin Endocrinol Metab. 57:262–267.
42. **Cohn JS, Wagner DA, Cohn SD, Millar JS, Schaefer EJ.** 1990 Measurement of very low density and low density lipoprotein apolipoprotein (apo) B-100 and high density lipoprotein apo A-I production in human subjects using deuterated leucine. J Clin Invest. 85:804–811.