Small Low-Density Lipoprotein Particles in Women With Natural or Surgically Induced Menopause

NOBUO IKENOUE, MD, AKIHIKO WAKATSUKI, MD, AND YUJI OKATANI, MD

***Objective*: To investigate the mechanism of the decrease in the size of low-density lipoprotein (LDL) particles in women with natural menopause and women with surgically induced menopause.**

***Methods*: We measured plasma levels of total cholesterol; triglycerides; high-density lipoprotein (HDL) cholesterol; apolipoproteins A-I, A-II, and B; and sex hormones in 45 women; 15 women were premenopausal, 15 were naturally postmenopausal, and 15 were surgically menopausal. Lipoprotein lipase and hepatic triglyceride lipase activities were measured in postheparin plasma. Concentrations of total cholesterol and of apolipoprotein B in LDL also were measured. Low-density lipoprotein particle diameter was determined by gradient gel electrophoresis.**

***Results*: Plasma levels of total cholesterol, triglycerides, apolipoprotein B, LDL-total cholesterol, LDL-apolipoprotein B, and the activity of postheparin plasma lipoprotein lipase were significantly higher and concentrations of estrone and estradiol were significantly lower in the naturally postmenopausal and surgically menopausal women than in the premenopausal women. Plasma levels of HDL cholesterol and apolipoproteins A-I and A-II and postheparin plasma hepatic triglyceride activity did not differ significantly between groups. The diameter of LDL particles was significantly reduced in the naturally (25.29 ± 0.19 nm) and surgically (25.29 ± 0.22 nm) menopausal women compared with the premenopausal women (25.88 ± 0.22 nm). Plasma triglyceride levels were negatively correlated with LDL particle diameter in all three groups (premenopausal group: $r = -0.64$, $P < .01$; naturally postmenopausal group: $r = -0.62$, $P < .01$; and surgically menopausal group: $r = -0.76$, $P < .001$). The prevalence of LDL subclass pattern B was significantly increased in the naturally (67%, $P < .05$) and surgically (60%, $P < .05$) menopausal women.**

***Conclusion*: The plasma concentration of LDL particles was increased after menopause, whether natural or surgically induced. An increase in plasma triglyceride levels in women with low levels of endogenous estrogen appeared to cause the size of LDL particles to be reduced. (Obstet Gynecol 1999;93:566–70. © 1999 by The American College of Obstetricians and Gynecologists.)**

Low-density lipoprotein (LDL), a major cholesterol-carrying lipoprotein in plasma, is implicated in the formation of atherosclerotic lesions.[1] The plasma level of LDL cholesterol increases after menopause,[2] and with age, women become more susceptible to coronary heart disease.[3] Premenopausal women who have undergone bilateral oophorectomies also have increased plasma LDL cholesterol levels and high incidences of coronary heart disease.[4] We previously demonstrated that a decrease in the plasma concentration of estrogen enhances lipoprotein lipase activity in naturally postmenopausal as well as in bilaterally oophorectomized women, which may lead to an increase in their plasma concentrations of LDL.[5] Arca et al[6] suggested that hypercholesterolemia in postmenopausal women is due to an impairment of their LDL receptors.

Low-density lipoprotein particles vary in size, density, and lipid composition,[7] with not all LDL subfractions being equally atherogenic. Smaller, denser LDL particles are associated with an increased risk of coronary heart disease.[8] Campos et al[9] reported that LDL particles are more likely to be smaller in postmenopausal than in premenopausal women, but the mechanism of this reduction has not been evaluated.

The size of LDL particles is closely related to plasma triglyceride levels,[10] with LDL particle diameter being smaller in patients with hypertriglyceridemia.[11] We previously demonstrated that an increase in plasma triglyceride levels, induced by estrogen therapy, appears to cause the size of LDL particles to be reduced.[12,13] Lipolytic enzymes such as lipoprotein lipase and hepatic triglyceride lipase enhance the production of LDL particles by catalyzing the hydrolysis of triglycerides in very low density lipoprotein and intermediate-density lipoprotein. Hepatic triglyceride lipase ac-

From the Department of Obstetrics and Gynecology, Kochi Medical School, Kochi, Japan.

celerates the conversion of LDL particles from large to small.[14] A decrease in the activity of hepatic triglyceride lipase results in the production of large LDL particles that are rich in triglycerides.[15] Therefore, these lipolytic enzymes also may affect the LDL subclass pattern by influencing the composition of LDL particles.

We measured plasma lipid levels, lipolytic enzyme activities, and the size of LDL particles in women with natural menopause and women with surgically induced menopause, making comparisons with premenopausal women, to determine the mechanisms of the reduction in size of LDL particles in women with low plasma estrogen levels.

Materials and Methods

Between April 1, 1996, and October 31, 1997, we studied 45 healthy Japanese women: 15 premenopausal women aged 30–45 years with regular menstrual cycles, 15 naturally postmenopausal women aged 49–64 years, and 15 women with surgically induced menopause aged 33–46 years. The latter patients had undergone bilateral ovariectomies before menopause. Fifteen subjects in each group were sufficient for detecting statistical differences among groups, according to the results of our previous studies,[5,12,13] and subjects were enrolled consecutively in each group. Written informed consent was obtained from each subject before admission to the study. The study design was approved by the ethics committee of Kochi Medical School. No subject was a smoker; ingested caffeine or alcohol; had a history of thyroid disease, liver disease, or diabetes mellitus; or currently was taking medication known to influence lipoprotein metabolism. None of the women were undergoing estrogen replacement therapy (ERT). None of the naturally postmenopausal subjects had had a menstrual period within the past year. The surgically menopausal subjects had undergone bilateral ovariectomies at least 2 years before participation in the study.

We obtained 12-hour fasting blood samples between 8:00 AM and 10:00 AM. In the premenopausal women, blood samples were drawn at the midfollicular phase of the menstrual cycle. Blood was centrifuged immediately at 1500 $\times$ *g* for 20 minutes at 4C to separate the plasma for assay of concentrations of total cholesterol, triglycerides, apolipoproteins, and sex hormones. After blood sampling, heparin (10 U/kg of body weight) was administered intravenously, and then 5.0 mL saline was infused to flush the line. Ten minutes later, blood was collected and postheparin plasma was obtained by centrifugation at 1500 $\times$ *g* for 20 minutes at 4C for assay of lipoprotein lipase and hepatic triglyceride lipase activity.[5]

Low-density lipoprotein subsequently was fractionated by ultracentrifugation according to the method of Havel et al.[16] Plasma levels of total cholesterol, triglycerides, and LDL cholesterol were measured enzymatically.[17] After the apolipoprotein B–containing lipoproteins were precipitated with sodium phosphotungstate in the presence of magnesium chloride, concentrations of high-density lipoprotein (HDL) cholesterol were measured enzymatically.[17] Plasma levels of apolipoproteins A-I, A-II, and B and the concentration of LDL–apolipoprotein B were measured using a turbidimetric immunoassay.[18] Plasma levels of estrone (E1) and estradiol (E2) were measured using radioimmunoassay kits (MSI; Bristol, Tokyo, Japan).

The activities of lipoprotein lipase and hepatic triglyceride lipase in postheparin plasma were determined as previously reported.[5]

Low-density lipoprotein particle diameters were determined by gradient gel electrophoresis, using 2–15% nondenaturing polyacrylamide-agarose gels as previously described.[19] The gels were stained with Coomassie G-250 (Nacalai, Kyoto, Japan). The distribution profile of the LDL subfractions was determined by densitometric scanning of the gels at 633 nm (Shimadzu, Kyoto, Japan). The apparent diameter of the major LDL subfractions was determined by comparing the results with a calibration curve, constructed with the use of ferritin, thyroglobulin, and latex beads as reference compounds.[19]

Data are reported as mean $\pm$ standard error. Differences between groups were analyzed by one-way analysis of variance. Regression lines were determined by the least squares method. If analysis of variance showed a significant difference, Scheffé multiple comparison procedure was applied to determine which values differed. Differences in the population of LDL subclass patterns were determined by χ^2 test. A level of $P < .05$ was accepted as statistically significant.

Results

Analysis of variance showed significant differences between groups in age; plasma levels of total cholesterol, triglycerides, apolipoprotein B, LDL cholesterol, and LDL apolipoprotein B; and diameter of LDL particles. Age and plasma levels of total cholesterol, triglycerides, apolipoprotein B, LDL cholesterol, and LDL apolipoprotein B in the naturally as well as in the surgically menopausal women were significantly greater than in the premenopausal women. The diameter of LDL particles was significantly reduced in both groups of menopausal women. No significant between-group differences were found in body mass index or plasma levels of HDL cholesterol and apolipoproteins A-I and A-II (Table 1).

Table 1. Subject Characteristics, Plasma Lipid and Lipoprotein Concentrations, Low-Density Lipoprotein Particle Diameters, Plasma Estrogen Levels, and Postheparin Plasma Lipolytic Enzyme Activities

	Premenopausal group (n = 15)	Naturally postmenopausal group (n = 15)	Surgically menopausal group (n = 15)	P
Age (y)	42.75 ± 1.87	56.33 ± 1.45*	43.67 ± 0.41†	<.001
Body mass index (kg/m^2)	22.52 ± 0.72	23.09 ± 0.67	23.52 ± 1.14	NS
Total cholesterol (mg/dL)	184.11 ± 10.75	269.25 ± 8.98*	240.31 ± 11.11‡	<.001
Triglycerides (mg/dL)	92.14 ± 8.27	125.16 ± 9.09§	124.49 ± 19.81§	<.05
HDL cholesterol (mg/dL)	59.68 ± 3.72	59.66 ± 2.84	62.49 ± 3.79	NS
Apolipoprotein A-I (mg/dL)	125.14 ± 8.62	134.77 ± 4.55	138.50 ± 5.44	NS
Apolipoprotein A-II (mg/dL)	34.40 ± 3.95	36.62 ± 1.01	39.60 ± 3.15	NS
Apolipoprotein B (mg/dL)	69.71 ± 3.95	122.70 ± 4.69§	112.45 ± 9.95§	<.05
LDL cholesterol (mg/dL)	100.29 ± 8.86	163.35 ± 7.28*	147.28 ± 5.58‡	<.001
LDL apolipoprotein B (mg/dL)	69.71 ± 3.34	122.70 ± 7.89*	112.45 ± 17.19‡	<.001
LDL particle diameter (nm)	25.88 ± 0.22	25.29 ± 0.19§	25.29 ± 0.22§	<.05
Estrone (pg/mL)	77.40 ± 10.02	32.00 ± 4.42‡	38.93 ± 4.83‡	<.01
Estradiol (pg/mL)	69.10 ± 13.26	15.40 ± 1.54*	17.96 ± 1.99*	<.001
Lipoprotein lipase activity (μmol/mL/h)	9.97 ± 1.01	13.56 ± 0.69§	13.72 ± 0.73§	<.05
Hepatic triglyceride lipase activity (μmol/mL/h)	15.11 ± 2.10	14.92 ± 1.59	15.27 ± 1.81	NS

NS = not significant; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
Data are expressed as mean ± standard error.
* $P < .001$, compared with the premenopausal group.
† $P < .001$ compared with the naturally postmenopausal group.
‡ $P < .01$ compared with the premenopausal group.
§ $P < .05$ compared with the premenopausal group.

Statistically significant differences in plasma levels of E1 and E2 were observed among the three groups. The plasma levels of E1 and E2 were significantly decreased in women with natural or surgically induced menopause compared with premenopausal subjects (Table 1). Although the between-group differences in the activity of postheparin plasma lipoprotein lipase approached statistical significance, no significant difference was found in the activity of postheparin plasma hepatic triglycerides. The activity of postheparin plasma lipoprotein lipase was significantly increased in both groups of menopausal women. The prevalence of LDL subclass pattern B was significantly greater in the naturally menopausal (67%) and the surgically menopausal (60%) women than in the premenopausal women (20%) (Table 2).

There was a significant negative correlation between LDL particle diameter and plasma triglyceride levels in all three groups. The diameter of LDL particles also was negatively correlated with plasma levels of total cholesterol in the premenopausal and the surgically menopausal women. The diameter of LDL particles was negatively correlated with plasma levels of LDL cholesterol in the premenopausal women and with plasma levels of apolipoprotein B in women with surgically induced menopause. A significant positive correlation was found between LDL particle diameter and plasma levels of HDL cholesterol in the surgically menopausal women. No other significant correlations were observed between plasma levels of other lipids evaluated and LDL particle diameter (Table 3).

Table 2. Prevalence of Low-Density Lipoprotein Subclass Patterns

Group	LDL subclass pattern A*/B† (no. of subjects)
Premenopausal	12/3
Naturally postmenopausal	5/10‡
Surgically menopausal	6/9‡

LDL = low-density lipoprotein.
* Diameter of LDL particle ≥ 25.5 nm.
† Diameter of LDL particle < 25.5 nm.
‡ $P < .05$ compared with the premenopausal group.

Discussion

Low-density lipoprotein levels, which play a major role in coronary heart disease, have been shown to increase after menopause.[2] In the present study, plasma levels of LDL–apolipoprotein B were increased in menopausal women, whether the menopause was naturally or surgically induced. Each LDL particle has one molecule of apolipoprotein B; thus, an increase in plasma levels of LDL–apolipoprotein B indicates that a decrease in plasma estrogen levels may increase plasma concentrations of LDL particles. Plasma levels of LDL in women with low plasma estrogen levels are increased by the decrease in the activity of hepatic LDL receptors[5] and by stimulation of the activity of lipoprotein lipase,[5] a

Table 3. Correlation Coefficients Between Low-Density Lipoprotein Particle Diameters and Plasma Lipid Levels

Variable	Menopause type	Correlation coefficient
Total cholesterol	Pre-	−0.51*
	Natural	−0.43
	Surgically induced	−0.57*
Triglyceride	Pre-	−0.64†
	Natural	−0.62†
	Surgically induced	−0.76‡
HDL cholesterol	Pre-	0.32
	Natural	0.25
	Surgically induced	0.57*
LDL cholesterol	Pre-	−0.56*
	Natural	−0.20
	Surgically induced	−0.43
Apolipoprotein A-I	Pre-	0.31
	Natural	0.23
	Surgically induced	0.28
Apolipoprotein A-II	Pre-	0.34
	Natural	0.43
	Surgically induced	0.47
Apolipoprotein B	Pre-	−0.16
	Natural	−0.48
	Surgically induced	−0.59*

HDL = high-density lipoprotein; LDL = low-density lipoprotein.
* $P < .05$.
† $P < .01$.
‡ $P < .001$.

key enzyme for LDL production. An elevation in LDL cholesterol concentrations may be accompanied by an increase in plasma concentrations of LDL particles.

Smaller, denser LDL particles are associated with an increased risk of coronary heart disease.[8] These small, dense LDL particles have a low affinity for the LDL receptor and thus are likely to accumulate in the bloodstream.[20] They also are more susceptible to oxidative modification,[21] an initial step in the atherosclerotic process. In the present study, gradient gel electrophoretic analysis revealed a reduction in the diameter of LDL particles in both groups of menopausal women.

The diameter of the LDL particles was positively correlated with plasma levels of HDL cholesterol in the surgically menopausal women and negatively correlated with plasma levels of total cholesterol in the premenopausal and in the surgically menopausal women. The LDL particle diameter also was negatively correlated with plasma levels of LDL cholesterol in the premenopausal women. In all three groups, however, plasma levels of triglycerides were strongly negatively correlated with LDL particle diameter. McNamara et al[10] suggested that plasma levels of triglycerides are the single most important influence on LDL particle size, with variations in plasma concentrations of triglycerides affecting particle size. We also previously reported that estrogen-induced increase in plasma levels of triglycerides causes reduction in the size of LDL particles in postmenopausal women.[12,13] In the present study, plasma triglyceride levels were increased in the naturally as well as in the surgically menopausal women, consistent with another report.[22] These findings suggest that an increase in plasma triglyceride levels induced by natural or surgical menopause may cause reduction in the size of LDL particles. Triglyceride and cholesteryl ester are the core lipids in the lipoprotein particles that are the major determinants of particle size. We previously reported that estrogen-induced hypertriglyceridemia may enhance lipid transfer reactions, resulting in small LDL particles that are rich in triglycerides and poor in cholesteryl ester.[13] Therefore, an increase in plasma levels of triglycerides in naturally or surgically menopausal women may affect the composition of LDL particles through lipid transfer reactions and result in the production of small LDL particles. However, enrichment of the LDL core with triglycerides did not promote the formation of small LDL particles.[23] The LDL particles tended to become larger because the volume of triglyceride molecules may exceed that of cholesteryl ester molecules.[24] A reduction in the size of LDL particles therefore may involve additional factors. Reduced activity of lipoprotein lipase is a component of the metabolic profiles associated with increased levels of small LDL particles.[25] In the present study, however, the activity of lipoprotein lipase was increased in both groups of menopausal women. This suggests that lipoprotein lipase may not be a major determinant of LDL particle size. The hydrolysis of LDL core triglycerides by such lipolytic enzymes as hepatic triglyceride lipase is accompanied by a reduction in LDL particle size.[14] A reduction in hepatic triglyceride lipase activity results in the production of large, triglyceride-rich LDL particles.[15] The activity of hepatic triglyceride lipase thus may influence the LDL subclasses. In this study, the activity of hepatic triglyceride lipase did not differ significantly between groups, consistent with our previous findings.[4] It therefore is unlikely that the size of LDL particles is reduced solely by the action of hepatic triglyceride lipase. These findings suggest that the size of LDL particles appears to be reduced by the combined effects of enhanced lipid transfer reactions and the hydrolysis of triglycerides by hepatic triglyceride lipase within the particles.

Two distinct LDL subclass patterns have been identified.[26] Pattern A consists of LDL particles with diameters of at least 25.5 nm, and pattern B consists of particles with diameters less than 25.5 nm.[8] Low-density lipoprotein subclass pattern B is associated with an increased risk of atherosclerosis. The present study showed that the prevalence of LDL subclass pattern B was increased after either natural or surgical menopause, which suggests that the

decrease in LDL particle diameter in women after menopause may be atherogenic.

Estrogen replacement therapy after natural or surgically induced menopause reduces the incidence of ischemic heart disease and the mortality rate associated with cardiovascular disease.[27,28] This antiatherogenic effect of estrogen may be related to a decrease in the number of LDL particles. However, we previously reported that the estrogen-induced increase in plasma triglyceride levels causes a reduction in the size of LDL particles and that the beneficial effects of estrogen could be offset in part by a concurrent reduction in LDL particle size.[13] Therefore, the small size of LDL particles in women with low plasma estrogen levels would be decreased further by estrogen therapy. Studies are needed to investigate whether women who are undergoing ERT after natural or surgically induced menopause require additional therapy to reduce plasma levels of triglycerides.

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Address reprint requests to:
Akihiko Wakatsuki, MD
Department of Obstetrics and Gynecology
Kochi Medical School
Oko cho, Nankoku
Kochi
Japan

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