Comparison of the Effects of Triphasic Oral Contraceptives With Desogestrel or Levonorgestrel on Apolipoprotein A-I–Containing High-Density Lipoprotein Particles

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Recent observations suggest that the risk of coronary artery disease (CAD) is associated with both the level and composition of the two major populations of apolipoprotein (apo)-defined high-density lipoprotein (HDL) particles: those containing both apo A-I and apo A-II [Lp(AI,AII)] and those containing apo A-I without apo A-II [Lp(AI)]. While sex hormones are known to affect HDL, their influence on these apo-defined HDL particles is not known. We have determined the effects of two triphasic oral contraceptive (OC) formulations on these HDL particles in healthy normolipidemic women aged 21 to 35 years. The formulations contain comparable quantities of ethinyl estradiol (EE) and either desogestrel (DG), a minimally androgenic progestin, or levonorgestrel (LN), a more androgenic progestin. Lipid and lipoprotein levels were measured during the third week of the normal menstrual cycle and the sixth month of OC use. The DG/EE formulation significantly increased total cholesterol (C) 15%, triglyceride (TG) 99%, phospholipid (PL) 17%, apo A-I 28%, apo A-II 34%, apo B 21%, very–low-density lipoprotein cholesterol (VLDL-C) 238%, HDL-C 20%, and HDL_3-C 28% ($P < .02$ to .005, n = 11), but not low-density lipoprotein cholesterol (LDL-C). The LN/EE formulation also increased total C 15%, TG 33%, apo A-I 15%, HDL_3-C 21% ($P < .05$, n = 10), apo B 30% ($P < .005$), and, additionally, LDL-C 19% ($P < .05$). Both formulations increased Lp(AI,AII) (DG/EE, 34%, $P < .005$; LN/EE, 24%, $P < .01$). These changes reflected comparable increases of small (7.0 to 8.2 nm) and medium (8.2 to 9.2 nm) particles in the LN/EE group and a predominant increase of medium-sized particles in the DG/EE group. Also, in the LN/EE group but not the DG/EE group, there were fewer large (9.2 to 11.2 nm) particles. Lp(AI) increased only in the DG/EE group (25%, $P = .075$) and was due to the presence of more large particles. The level of Lp(AI) did not change in the LN/EE group, but the lipid/A-I ratio of these particles was lower ($P = .012$) and there were more small particles. Thus, triphasic OC formulations with progestins of different androgenicity had different effects on VLDL, LDL, and the level and composition of HDL particles with and without apo A-II, possibly reflecting estrogen/progestin/androgen balance. Estrogen dominance increases both Lp(AI,AII) and Lp(AI) and favors large Lp(AI) particles, while progestin/androgen dominance increases only Lp(AI,AII) and favors small particles. Because of the importance of HDL in the arterial wall physiology, OC formulations with different estrogen and progestin content may affect arterial wall health to a different extent.

HUMAN PLASMA high-density lipoproteins (HDLs) are composed of a heterogeneous population of particles differing in their physical and chemical properties. On the basis of flotation rates, these lipoproteins are conventionally divided into two subfractions, HDL_2 and HDL_3.[1] On the basis of apolipoprotein (apo) content, two major populations of particles have been identified: those containing both apo A-I and A-II [Lp(AI.AII)] and those containing apo A-I but not A-II [Lp(AI)].[2-4] Both populations of particles are present in HDL_2 and HDL_3,[4-6] and contain size subspecies with two, three, or four molecules of apo A-I per particle.[6] The levels and characteristics of these HDL particles differ between healthy normolipidemic individuals and those with documented coronary artery disease (CAD) or at risk for CAD.[7-11] They are catabolized at different rates[12-14] and have been shown to respond differently to dietary and pharmacologic perturbations,[15-17] suggesting that they are distinct metabolic entities.

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Epidemiological studies have repeatedly demonstrated an inverse relationship between HDL levels and the risk of CAD.[18] Sex hormones are known to affect HDL levels. Estrogens increase and androgens decrease HDL, while progestogen-induced changes vary with the androgenicity of the compound.[19-23] Their effects on the apo-defined HDL particles are not known. Since HDL particles with and without apo A-II are metabolically distinct and gender differences in these particles have been reported,[24] we hypothesize that they may respond differently to hormonal perturbations. To test this hypothesis, we determined the effects of two oral contraceptive (OC) formulations with similar estrogen content but with progestins of different androgenicity on the level and composition of these HDL particles.

SUBJECTS AND METHODS

Subjects and Study Design

The subjects were 21 healthy normolipidemic women aged 21 to 35 years with regular menstrual cycles (25 to 35 days). They were a randomly selected subset of 60 volunteers who participated in an open-label, comparative lipid profile study of two triphasic OC formulations containing comparable quantities of ethinyl estradiol (EE) and either desogestrel (DG), a minimally androgenic progestogen (DG/EE, 7 days 50 μg/35 μg, 7 days 100 μg/30 μg, and 7 days 150 μg/30 μg; CTR-05; Organon, West Orange, NJ), or levonorgestrel (LN), a more androgenic progestogen (LN/EE, 6 days 50 μg/30 μg, 5 days 75 μg/40 μg, and 10 days 125 μg/30 μg; TriphasilR-21, Wyeth-Ayerst Laboratories, Philadelphia, PA). The DG/EE formulation is presently available only in Europe. The subjects were nonsmokers with no demonstrated evidence of thyroid, hepatic, renal, gastrointestinal,

gynecologic, hematologic, neurologic, respiratory, endocrine, or cardiovascular abnormality and were not on lipid-lowering medications or other agents known to affect lipid metabolism, nor had they used OCs within 90 days prior to the study. After completing all screening procedures, subjects were randomly assigned to receive either the DG/EE formulation (n = 11) or the LN/EE formulation (n = 10). Lipoproteins in 12 to 14-hour fasting plasma samples were studied in the third week of the normal menstrual cycle (baseline) and the sixth month of contraceptive use. This study was approved by the University of Washington Human Subjects Review Committee, and informed consent was obtained from all subjects before entering the study.

Lipoprotein Fractionation

Fractionation of the plasma into very–low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and HDL was performed as previously described.[25] Briefly, the plasma was subjected to ultracentrifugation at 105,000 × *g* for 18 hours without prior density adjustment. VLDL was recovered in the top fraction by the tube-slicing technique. LDL and HDL were recovered in the bottom fraction. HDL was separated from the plasma by precipitation with dextran sulfate and magnesium.[26] The lipid in each fraction was quantified. LDL lipid was calculated as the difference between the d < 1.006 bottom fraction and the dextran sulfate–magnesium (DS-Mg) supernatant fraction. VLDL lipid was calculated as the difference between plasma lipid and the d < 1.006 bottom fraction.

Isolation of HDL Particles

All plasma samples used for the isolation of apo-defined HDL particles were absorbed with dextran sulfate cellulose to remove apo B–containing lipoproteins.[27,28] Lp(AI,AII) and Lp(AI) were isolated from apo B–free plasma by a previously established two-step immunoaffinity chromatographic procedure using antibodies specific for apo A-I and A-II.[2] Lipoproteins were eluted from the immunosorbents with 3 mol/L sodium thiocyanate in 0.01 mol/L sodium phosphate buffer, pH 7.0, and immediately desalted with a column of Sephadex G25 (Pharmacia-LKB Biotechnology, Uppsala, Sweden) and concentrated for lipid, apolipoprotein, and size analyses. All isolation and concentration processes were performed at 4°C.

Analytical Procedures

Total cholesterol (C), free cholesterol (FC), phospholipid (PL), and triglyceride (TG) levels in plasma and lipoprotein fractions were measured by enzymatic methods.[29] Cholesteryl ester (CE) mass was calculated as the difference between total C and FC with adjustment for the differences in the molecular weight of FC (387) and CE (650). All lipid analyses were performed on a Spectrum Analyzer (Abbott Laboratories, Chicago, IL). Cholesterol in HDL, HDL_2, and HDL_3 was determined by a two-step dextran sulfate–magnesium precipitation method.[26] Apo A-I and A-II were quantified by specific immunoassays.[30] The apo B level was measured with a Behring nephelometer using Behring reagents (Behring Diagnostic, Somerville, NJ) and calibrated with the Northwest Lipid Research Laboratory calibrators. The distribution of plasma apo A-I between Lp(AI,AII) and Lp(AI) was determined by quantifying the apo A-I in the isolated lipoproteins with proper adjustment for recovery. (Recoveries of apo A-I in the 21 samples at baseline, 96.1% ± 9.17%, and after OC use, 95.9% ± 9.74%, were comparable.) HDL particle sizes were determined by nondenaturing gradient polyacrylamide gel electrophoresis (gPAGE) using precast 4% to 30% gels (Pharmacia-LKB Biotechnology) and thyroglobulin, apoferritin, lactate dehydrogenase, and bovine albumin as calibration proteins.[8,31] Proteins in the gels were visualized with 0.1% Coomassie blue G-250. The 4% to 30% gels were scanned with a laser densitometer and integrated with the LKB 2400 GelscanXL software as previously described.[8] Briefly, the total integrated area of each densitometric scan between 7.0 and 17.0 nm (the largest and smallest referenced proteins) was considered to be 100%. Perpendicular lines were drawn from the positions corresponding to the Stokes diameters 7.0, 8.2, 9.2, 11.2, and 17.0 nm. These size intervals were chosen based on the clustering of particles found in healthy normolipidemic subjects. The ratio of the area between two perpendicular lines to the total area of the scan represents the relative proportion of the particles within that size interval. The concentration of apo A-I + A-II and apo A-I in HDL particles with and without apo A-II, respectively, in each size interval was calculated by multiplying the plasma concentration of apo A-I and A-II associated with these particles by the percentage of particles in each size interval.

Statistical Analyses

Nonparametric statistical tests were used because of the small sample size and skewing of some of the data. The lipid, lipoprotein, and apolipoprotein values at baseline and after 6 months of OC use were compared within each of the two OC groups by the Wilcoxon matched-pairs test. Between-group comparisons of the baseline to 6-month changes were made using the Mann-Whitney test.[32] Several levels of significance are provided; they are from two-tailed tests, and have not been adjusted for multiple comparisons.

RESULTS

Effects on Plasma Lipids, Apolipoproteins, and Lipoproteins

The mean ages of the two groups of subjects receiving DG/EE and LN/EE were nearly identical, 28.8 ± 3.3 and 28.3 ± 2.8 years, respectively. Their plasma lipid and apo A-I, A-II, and B levels at baseline were comparable (Table 1). The DG/EE formulation significantly increased all lipid and protein parameters measured except LDL-C and HDL_2-C. In the LN/EE group, significant increases were found for plasma C, TG, apo A-I, apo B, LDL-C, and HDL_3-C and a marginal increase in apo A-II ($P = .059$) was detected. When the changes between the two groups were compared, increases in plasma TG, apo A-II, VLDL-C, and HDL_2-C were greater in the DG/EE group (Table 1 and Fig 1). The percent changes in apo A-I and HDL-C were also higher with DG/EE, although they were not statistically significant. The difference in the HDL_2-C changes between the two OC groups is due to both a slight increase of this fraction with DG/EE and a slight decrease with LN/EE. The greater increase of HDL-C with no change in LDL-C in the DG/EE group also resulted in a significant increase in the calculated HDL-C:LDL-C ratio from 0.68 to 0.81. In contrast, LDL-C increased more than HDL-C in the LN/EE group (19% *v* 9%), slightly decreasing the calculated HDL-C:LDL-C ratio from 0.60 to 0.56.

Effect on HDL Particles

The level, composition, and size profile of HDL particles with or without apo A-II at baseline were comparable between the two groups of subjects (Tables 2 and 3). Both OC formulations increased the apolipoprotein and lipid content of Lp(AI,AII). The changes were generally greater in the DG/EE group, with differences reaching statistical significance for apo A-II ($P = .012$) and TG ($P = .002$). When these particles were resolved into size subspecies by nondenaturing gPAGE, a relative shift to a smaller size profile was observed in both groups. Figure 2 illustrates the change in the apo A-I + A-II

Table 1. Plasma Lipid and Apolipoprotein Levels (mean ± SD) at Baseline and 6 Months After Using One of Two OCs and the Percent Change From Baseline to 6 Months

Plasma Lipid or Protein (mg/dL)	DG/EE (n = 11)			LN/EE (n = 10)			P§
	Baseline	6 Months	% Change	Baseline	6 Months	% Change	
Cholesterol	170 ± 23	197 ± 37†	15 ± 14 (13)	167 ± 21	190 ± 28*	15 ± 20 (12)	.597
TG	58 ± 21	111 ± 37‡	99 ± 43 (81)	60 ± 23	75 ± 22*	33 ± 41 (11)	.002
PL	210 ± 18	245 ± 27‡	17 ± 10 (13)	201 ± 32	218 ± 26	10 ± 15 (10)	.158
Apo A-I	144 ± 17	182 ± 16‡	28 ± 15 (27)	134 ± 22	153 ± 26*	15 ± 17 (18)	.066
Apo A-II	31 ± 4	41 ± 4†	34 ± 22 (37)	32 ± 5	35 ± 4	11 ± 16 (10)	.012
Apo B	80 ± 16	98 ± 28*	21 ± 20 (27)	81 ± 11	105 ± 27‡	29 ± 28 (25)	.724
Cholesterol content							
VLDL	8 ± 6	16 ± 10†	238 ± 341 (120)	10 ± 6	12 ± 4	76 ± 161 (19)	.022
LDL	99 ± 22	104 ± 33	4 ± 21 (4)	98 ± 13	116 ± 26*	19 ± 31 (13)	.217
HDL	64 ± 8	77 ± 12‡	20 ± 12 (18)	58 ± 13	62 ± 15	9 ± 17 (15)	.061
HDL_2	25 ± 6	27 ± 9	9 ± 22 (8)	21 ± 9	18 ± 8	−10 ± 25 (0)	.037
HDL_3	39 ± 4	50 ± 6‡	28 ± 15 (24)	37 ± 6	44 ± 8*	21 ± 18 (25)	.392
HDL-C/LDL-C	0.68 ± 0.17	0.81 ± 0.29†	0.14 ± 20 (0.11)‖	0.60 ± 0.15	0.56 ± 0.17	−0.04 ± 0.12 (0.02)‖	.024

NOTE. Numbers in parentheses are the median.

*$P \leq .05$, †$P \leq .01$, and ‡$P \leq .005$ *v* baseline by Wilcoxon matched-pairs signed-rank test.

§Mann-Whitney test comparing baseline and 6-month change in DG/EE *v* LN/EE groups.

‖Absolute change, not percent change.

concentration in each of the size subspecies. In the DG/EE group, this shift was entirely due to the increase of Lp(AI,AII) in the medium (8.2 to 9.2 nm) and small (7.0 to 8.2 nm) size intervals. There was no detectable change in the concentration of apo A-I + A-II in the large (9.2 to 11.2 nm) particles. In the LN/EE group, this shift reflects both a decrease of the apo A-I + A-II concentration in particles larger than 9.2 nm and an increase in particles smaller than 9.2 nm.

Differences in the Lp(AI) response to DG/EE and LN/EE were also found. With DG/EE (but not LN/EE), the apo A-I and TG content in these particles increased 25% ($P = .075$) and 69% ($P = .002$), respectively (Table 3). The moderate increase in total apo A-I was the result of an increase in the apo A-I concentration of larger particles, those greater than 9.2 nm in Stokes diameter (Fig 3). Although no change in the level of Lp(AI) was found in the LN/EE group, the mean concentration of all lipid components measured decreased slightly at the 6-month follow-up study. While none of the individual lipid changes were statistically significant ($P = .203$ to $.541$), cumulatively, they reflect particles with significantly lower lipid content. The calculated lipid/A-I ratio decreased from 1.36 ± 0.22 at baseline to 1.07 ± 0.24 after LN/EE use ($P = .012$). Size profile analyses showed a decrease of large (9.2 to 11.2 nm) particles ($P = .025$) and a reciprocal increase of small (7.0 to 8.2 nm) particles ($P = .005$) (Table 3). These changes in the size profile and TG content of these particles were significantly different between the two groups.

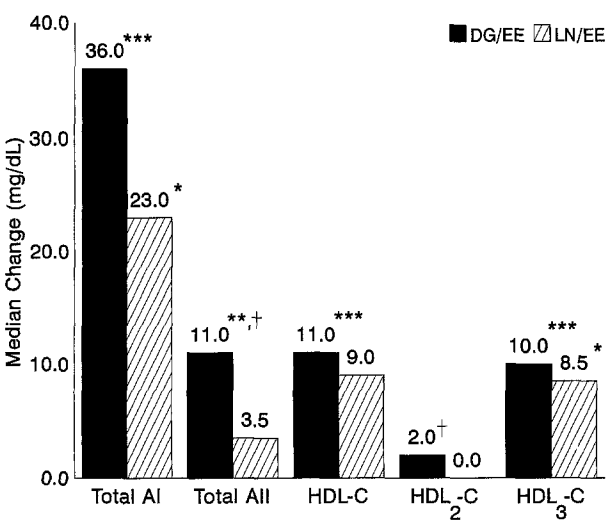

Fig 1. Changes in HDL parameters associated with triphasic DG/EE and LN/EE OC formulations. *$P \leq .05$, **$P \leq .01$, and ***$P \leq .005$ *v* baseline. †$P \leq .05$, DG/EE *v* LN/EE.

DISCUSSION

Estrogen is associated with numerous beneficial effects on lipoprotein metabolism. Among these are an enhanced clearance of remnant lipoproteins[33,34] and LDL[35] due to an increase in LDL receptor activity[36] (see Knopp et al[37,38] for review). An increase in HDL concentrations with an increase in apo A-I and HDL_2 is also described with estrogen therapy.[39] All of these effects are considered beneficial in preventing CAD.[22,38]

On the other hand, combined estrogen-progestin administration is associated with an increase in HDL_3 and variable changes in HDL_2.[21] Depending on the androgenicity of the progestin, an increase in LDL[21,40] and potentially negative effects on atherosclerosis[41] have been observed. The influence of estrogens and progestins on HDL metabolism is potentially important because of the key role of HDL in reverse cholesterol transport. Despite the interest and importance of these hormone-mediated effects, it is not known precisely how the hormones affect the reverse cholesterol transport process. Among the elements of this process are the uptake of FC and PL from cells by an apo A-I–mediated process,[42] esterification of cholesterol, and transport of cholesterol through a series of enlarging HDL particles. Some of these apo A-I–containing particles of increasing size and buoyancy contain apo A-II, and some do not. A reduction of apo A-I particles without apo A-II and a predomi-

Table 2. Characteristics of Lp(AI, AII) at Baseline and 6 Months After Using One of Two OCs and the Percent Change From Baseline to 6 Months

Apolipoprotein and Lipid (mg/dL)	DG/EE (n = 11)			LN/EE (n = 10)			
	Baseline	6 Months	% Change	Baseline	6 Months	% Change	P§
Apo A-I	87.1 ± 15.4	113.8 ± 11.7‡	34 ± 23 (29)	84.4 ± 13.0	103.2 ± 14.6†	24 ± 19 (20)	.170
Apo A-II	30.9 ± 4.0	40.7 ± 4.1†	34 ± 22 (37)	31.9 ± 4.6	35.0 ± 4.0	11 ± 16 (10)	.012
FC	8.7 ± 1.8	11.4 ± 3.1‡	32 ± 23 (29)	8.2 ± 1.7	11.2 ± 4.0*	44 ± 68 (20)	.503
CE	53.8 ± 6.6	65.4 ± 8.6†	22 ± 17 (23)	53.1 ± 9.0	60.1 ± 13.3*	14 ± 21 (15)	.291
TG	5.3 ± 2.3	10.7 ± 3.4‡	116 ± 70 (129)	5.2 ± 1.7	7.0 ± 2.0‡	38 ± 25 (40)	.002
PL	80.3 ± 9.7	105.3 ± 11.2‡	32 ± 17 (28)	80.2 ± 12.4	92.5 ± 20.7	18 ± 31 (17)	.067
Lipid/(A-I + A-II)	1.27 ± 0.13	1.25 ± 0.14		1.28 ± 0.22	1.25 ± 0.14		.999
Size interval, Stokes diameter, nm (%)							
7.0-8.2	12.3 ± 1.8	16.4 ± 4‡		12.8 ± 2.7	18.9 ± 4.7‡		.086
8.2-9.2	47.6 ± 5.8	51.6 ± 4.8*		45.6 ± 5.8	50.9 ± 5.6*		.524
9.2-11.2	34.4 ± 5.6	26.6 ± 5.7‡		32.7 ± 6.0	23.9 ± 6.3‡		.943
11.2-17.0	5.7 ± 1.4	5.4 ± 0.8		8.9 ± 5.9	6.3 ± 1.6		.638

NOTE. Numbers in parentheses are the median.
*$P \leq .05$, †$P \leq .01$, and ‡$P \leq .005$ *v* baseline by Wilcoxon matched-pairs signed-rank test.
§Mann-Whitney test comparing baseline and 6-month change in DG/EE *v* LN/EE groups.

nance of smaller particles of both apo-defined HDL subclasses have been found in patients with cardiovascular disease[7,8] or those at risk for CAD.[9-11]

In the course of studying the metabolic effects of two OC formulations, one estrogen-dominant (DG/EE) and the other progestin-dominant (LN/EE), we determined if there were associated alterations in the level and composition of the apo-defined HDL subclasses. We also wished to determine if the changes in subjects treated with the progestin-dominant formulation bore any similarity to the HDL subclass changes associated with vascular disease or conditions associated with the risk for vascular disease.[7-11] We observed the differences in the major lipoprotein fractions expected in subjects taking estrogen-dominant versus progestin-dominant OC formulations. The estrogen-dominant form (DG/EE) was associated with higher TG, lower LDL-C, and higher HDL-C and HDL_2-C levels compared with the progestin-dominant form (LN/EE), as previously observed.[43,44] Both formulations also increased HDL_3-C levels, consistent with the previously reported effects of these two progestins when given with EE in triphasic formulations.[44]

The heterogeneity of HDL particles in the conventional density subclasses, HDL_2 and HDL_3, has been classified into a range of Stokes diameters as determined by gPAGE: HDL_{2b} (9.71 to 12.9 nm), HDL_{2a} (8.77 to 9.71 nm), HDL_{3a} (8.17 to 8.77 nm), HDL_{3b} (7.76 to 8.17 nm), and HDL_{3c} (7.21 to 7.76 nm).[30] The two apo-defined HDL subclasses, Lp(AI) and Lp(AI,AII), are present in varying quantities in all of these size subclasses.[6] The present study provides new information on the response of these HDL particles to the two OC formulations used. First, the estrogen-dominant DG/EE formulation increased both Lp(AI,AII) and Lp(AI) levels, while the progestin-dominant LN/EE increased only Lp(AI,AII) (Figs 2 and 3). Second, the increase of Lp(AI) induced by DG/EE occurred mostly among large particles greater than 9.2 nm in Stokes diameter, corresponding to HDL_{2a} and HDL_{2b}. Conversely, the increase in HDL particles containing apo A-II induced by both OC formulations was confined to the size regions smaller than 9.2 nm (Fig 2). Thus, the increases in HDL_2-C and HDL_3-C observed were primarily due to changes in Lp(AI) and Lp(AI,AII), respectively. Third, although both OC formulations increased Lp(AI,AII), their responses differed with respect to the size species. With LN/EE, similar increases were observed in the 7.0 to 8.2 nm and 8.2 to 9.2 nm regions. On the other hand, with DG/EE, the increase of particles in the 8.2 to 9.2 nm

Table 3. Characteristics of Lp(AI) at Baseline and 6 Months After Using One of Two OCs and the Percent Change From Baseline to 6 Months

Apolipoprotein and Lipid (mg/dL)	DG/EE (n = 11)			LN/EE (n = 10)			
	Baseline	6 Months	% Change	Baseline	6 Months	% Change	P§
Apo A-I	56.6 ± 10.4	68.2 ± 11.6*	25 ± 37 (21)	50.3 ± 16.3	49.6 ± 14.5	6 ± 34 (11)	.231
FC	5.0 ± 1.7	6.0 ± 2.3	22 ± 45 (8)	4.2 ± 1.9	3.5 ± 2.1	−10 ± 33 (3)	.159
CE	30.1 ± 9.1	31.6 ± 10.1	7 ± 28 (−2)	25.6 ± 12.2	19.6 ± 11.4	−14 ± 38 (−20)	.205
TG	3.8 ± 1.2	6.1 ± 2.0‡	69 ± 55 (48)	3.5 ± 1.8	2.9 ± 0.8	3 ± 48 (−9)	.001
PL	42.9 ± 9.9	49.7 ± 15.2	18 ± 37 (10)	36.6 ± 14.1	29.2 ± 15.9	−12 ± 39 (−6)	.098
Lipid/A-I	1.44 ± 0.21	1.35 ± 0.28		1.36 ± 0.22	1.07 ± 0.24†		.139
Size interval, Stokes diameter, nm (%)							
7.0-8.2	12.3 ± 2.5	12.2 ± 3.6		11.5 ± 3.1	17.0 ± 4.0‡		.007
8.2-9.2	28.9 ± 7.2	26.6 ± 5.5		25.6 ± 5.4	24.5 ± 4.8		.698
9.2-11.2	46.4 ± 6.6	47.4 ± 7.0		43.0 ± 8.7	38.2 ± 6.3†		.013
11.2-17.0	12.4 ± 3.0	13.8 ± 2.3		20.0 ± 11 5	20.3 ± 5.6		.306

NOTE. Numbers in parentheses are the median.
*$P = .075$, †$P < .05$, and ‡$P \leq .005$ *v* baseline by the Wilcoxon matched-pairs signed-rank test.
§Mann-Whitney test comparing baseline and 6-month change in DG/EE *v* LN/EE groups.

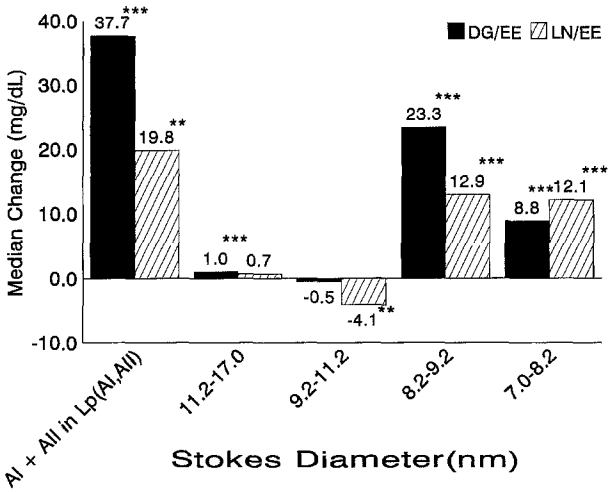

Fig 2. Changes in apo A-I + A-II levels in Lp(AI,AII) and its size subspecies with triphasic DG/EE and LN/EE OC formulations. **$P \leq .01$ and ***$P \leq .005$ *v* baseline. Stokes diameter is the diameter of HDL calculated from its diffusion coefficient in gradient gel.

region was nearly three times greater than the increase in the 7.0 to 8.2 nm region. This observation indicates that estrogen favors the increase of Lp(AI,AII) corresponding to HDL_{2a} and HDL_{3a}, whereas progestin has a more dominant effect on particles in the HDL_{3b} and HDL_{3c} sizes. Fourth, although no statistically significant reduction in HDL_2-C was detected in the group taking LN/EE, there were significantly fewer Lp(AI,AII) particles in the 9.2 to 11.2 nm (HDL_{2a} and HDL_{2b}) region (Fig 2). Similarly, no reduction was found in Lp(AI) levels in the LN/EE group, but this HDL subclass became relatively lipid-poor (Table 3) and enriched with small lipoproteins (Fig 3). This observation further supports the idea that androgenic progestins favor the formation of small dense HDL particles. Thus, the isolation and characterization of apo-defined HDL particles by

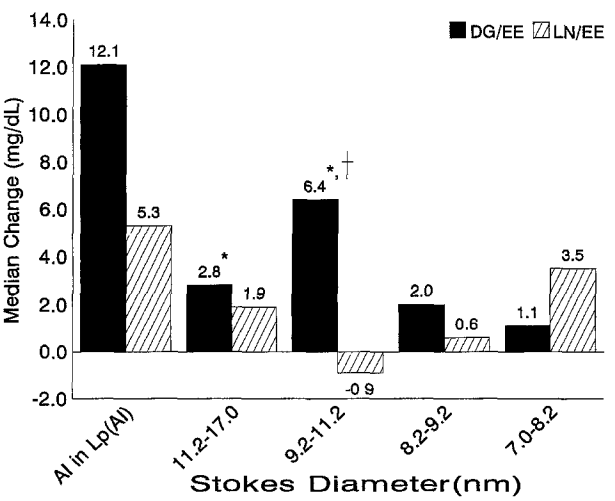

Fig 3. Changes in apo A-I levels in Lp(AI) and its size subspecies with triphasic DG/EE and LN/EE OC formulations. *$P \leq .05$ *v* baseline. †$P \leq .01$, DG/EE *v* LN/EE. Stokes diameter is the diameter of HDL calculated from its diffusion coefficient in gradient gel.

immunoaffinity chromatography and gradient gel electrophoresis provides a sensitive means of detecting HDL changes that are not revealed by conventional ultracentrifugation or precipitation methods. We have shown in a previous study that increased small dense HDL is associated with small dense LDL (LDL phenotype B).[45] Whether the LN/EE formulation also affected LDL particle density and size remains to be determined.

The response of HDL particles to OC formulations may reflect the influence of these hormones on lipoprotein synthesis and catabolism. The TG enrichment in Lp(AI,AII) with both formulations and in Lp(AI) with DG/EE is likely the result of estrogen-induced enhanced VLDL entry into the circulation and subsequent increased TG transfer from VLDL to these particles by the lipid transfer proteins.[37,38] The accumulation of smaller HDL particles with and without apo A-II in the LN/EE group, but not the DG/EE group, may be due to the different effects of these formulations on hepatic lipase (HL), which hydrolyzes HDL TG and promotes the formation of smaller HDL.[46] Estrogen reportedly decreases HL activity in postheparin plasma,[47,48] while LN increases HL activity.[44,49] In the same studies, DG had little or no effect on HL activity.[44,49] A relatively higher HL activity with the progestin-dominant LN/EE versus the estrogen-dominant DG/EE could explain the increased number of very small HDL particles observed with LN/EE but not with DG/EE. In contrast, the DG/EE formulation selectively increased large Lp(AI) particles. Since triphasic DG/EE had little effect on HL activity,[44] the known effect of estrogen to promote apo A-I production may be the responsible mechanism. Levels of Lp(AI)[3,50] and HDL_{2b}[31] are higher in females than in males. These gender differences have always been interpreted to be sex hormone–related. We have shown that nascent Lp(AI)s are either small spherical or large discoidal particles.[51] It has been suggested that the large discoidal particles are the precursors of HDL_{2b}.[52] We postulate that the estrogen-dominant DG/EE increased the production of large discoidal nascent Lp(AI) particles, resulting in the selective increase of these particles in the HDL_{2b} size range.

Some HDL characteristics that have been associated with CAD and/or the severity and progression of arteriosclerotic lesions include low HDL-C,[18] an elevated level of particles smaller than 8.2 nm,[8] or HDL_{3b} and HDL_{3c},[53] a reduced level of particles in the 9.2 to 11.2 nm interval,[8] or HDL_{2b},[53] and a low level of Lp(AI).[7] With the exception of a slight increase of small Lp(AI,AII) particles, the HDL changes observed with DG/EE are unlike those associated with atherosclerosis. Conversely, the progestin-dominant LN/EE formulation is associated with changes in HDL particle sizes associated with increased CAD risk, although the plasma Lp(AI) level was not significantly reduced. The presence of analogous effects in HDL subclasses of progestin-treated and atherosclerosis-prone individuals does not establish a causal relationship, but it is noteworthy that medroxyprogesterone acetate (MPA) nullified the antiatherosclerotic effect of estrogen in cholesterol-fed monkeys.[54] Likewise, in a large randomized trial, treatment with estrogen plus MPA did not reduce the overall rate of CAD events in postmenopausal women with CAD,[55] although unopposed estrogen has

been shown to have cardiovascular benefits in observational studies.[22,37]

In conclusion, differences in estrogen/progestin dominance in two triphasic OC formulations are associated with differences in apo-defined HDL particles. Estrogen dominance increases particles with and without apo A-II and favors larger Lp(AI), while progestin/androgen dominance increases only Lp(AI,AII) and favors smaller particles. Further studies are needed to determine the effects of these differences on reverse cholesterol transport, and on the efficacy of these HDL particles to promote cell cholesterol efflux.

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