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# PARTICLE SIZE INTERCONVERSION OF HUMAN LOW DENSITY LIPOPROTEINS DURING INCUBATION OF PLASMA WITH PHOSPHATIDYLCHOLINE VESICLES

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SUMMARY: Incubation of plasma (37°C, 6hr) in the presence of increasing amounts of phosphatidylcholine (PC) vesicles, above a threshold concentration, results in an increase in particle diameter of LDL relative to that from nonincubated plasma. With further PC addition, the major peak of LDL in the gradient gel electrophoretic pattern is transformed, first, into a bimodal and, subsequently, into a single peak distribution. PC-induced interconversion of LDL requires factor(s) in the $d > 1.20$ g/ml fraction and, at PC concentrations below approximately 2 mg/ml, is not inhibited by p-chloromercuriphenylsulfonic acid. Plasma incubation with increasing PC levels also leads to characteristic particle size transformations in $HDL_3$ species, with the transformation products ultimately converging to form a single peak pattern within the $HDL_{2a}$ size interval. In certain subjects, incubation of plasma, in the absence of added PC, shifts the particle size distribution of LDL towards smaller species; this can be prevented by addition of PC. We propose that incubation-induced shifts of LDL towards larger or smaller species result from changes in phospholipid (PL) content of LDL.

## INTRODUCTION

A number of discrete subpopulations within human plasma HDL (1) and LDL (2) classes have been recently demonstrated according to particle size by means of GGE. Interconversions among such subpopulations may contribute to the observed multicomponent distributions (3). One likely process contributing to interconversion involves interaction of lipoprotein species with surface components (PL, UC, apolipoproteins) released during lipolytic degradation of triglyceride-rich lipoproteins (chylomicrons or VLDL) (4). Evidence for the occurrence of such an interaction in association with interconversion of small HDL to larger species has been obtained *in vivo* (4) and *in vitro* (5,6,7).

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; PL, phospholipid; UC, unesterified cholesterol; VLDL, very low density lipoproteins; PC, phosphatidylcholine; merthiolate, ethyl mercurithiosalicyclic acid; PCMPS, p-chloromercuriphenylsulfonic acid; LCAT, lecithin:cholesterol acyltransferase; EDTA, ethylenediaminetetraacetic acid; Tris, tris (hydroxymethyl) aminomethane; GGE, gradient gel electrophoresis; CE, cholesteryl ester; DPPC, dipalmitoyl phosphatidylcholine.

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We have previously shown that incubation of isolated LDL with increasing concentrations of PC vesicles leads to gross aggregation of the LDL, but without detectable change in LDL particle size (8). In this report we describe conversion of LDL to larger species, when plasma is incubated in the presence of added PC.

## MATERIALS AND METHODS

Plasma from fasting subjects, one female (RM) and two males (EB and JG), was obtained as previously described (8). Merthiolate (0.5 mg/ml) and 5 microliter/ml penicillin-streptomycin (10,000 units/ml-10,000 microgram/ml; Gibco Laboratories) were immediately added to all plasmas. The plasma triglyceride levels (Triglyceride Reagent Set, Boehringer-Mannheim) in these subjects were 109 (RM), 197 (EB) and 550 (JG) mg/dl; the plasma HDL concentrations were 351 (RM), 196 (EB), and 200 (JG) mg/dl.

Incubation (37°C, 6hr) of plasma or lipoproteins with PC (total incubation volume, 3ml) was performed under $N_2$, in tightly-capped glass vials, in the presence or absence of 2mM PCMPS (Sigma Chemical Co.), used as an LCAT-inhibitor. Standard ultracentrifugal techniques (9) were used to isolate d 1.019-1.063 g/ml (LDL), d 1.063-1.20 g/ml (HDL), and d < 1.20 g/ml (total lipoprotein) fractions. In cases where the d < 1.20 g/ml fraction was used, the background solution (adjusted by dialysis prior to incubation) was 10mM Tris, 195mM NaCl, 0.27mM $Na_2EDTA$, 0.12mM merthiolate, pH 7.4. Aliquots were diluted to plasma concentration using the dialysis buffer.

Vesicles of egg yolk PC (US Biochemicals) were prepared according to a sonication method previously described (10). A few large, multilamellar structures were present in the vesicle preparations, as determined by negative stain electron microscopy (11). The stock vesicle concentration was 17.7 mg PC/ml in dialysis buffer.

Electrophoresis of d < 1.20 g/ml fractions obtained from plasma incubation mixtures was performed on PAA 2/16 and PAA 4/30 polyacrylamide gradient gels (Pharmacia Fine Chemicals) according to procedures previously described (1,2). A calibration mixture (latex beads, thyroglobulin, apoferritin) was run with every 2/16 gel and was used to establish the relationship between migration distance, relative to thyroglobulin, and particle diameter. GGE patterns were obtained by densitometry of the gels (protein stain; Coomassie G250) at 596nm. The variation in size measurements was about 2-3 Å, as previously reported (2).

Protein and phosphorus were measured according to the methods of Lowry et al. (12) and Bartlett (13), respectively. Total and unesterified cholesterol were determined enzymatically (Cholesterol Reagent Set, Boehringer-Mannheim).

## RESULTS AND DISCUSSION

The GGE profile of LDL obtained from subjects EB (Fig. 1A, lower inset) and JG (Fig. 1B, lower inset) showed major components of sizes 246 Å (size group E)[1] and 243 Å (size group F), respectively. Subject EB's plasma also had a minor LDL subpopulation (256 Å, size group B). Incubation of these plasmas alone produced little change in the size of the major LDL species. Incubation in the presence of increasing PL concentrations (in the form of PC vesicles) resulted in a characteristic sequence of changes in LDL properties (Fig. 1). Up to a threshold PC level

1. LDL size groups (A: 272-259 Å; B: 259-255 Å; C: 255-251 Å; D: 251-246 Å; E: 246-243 Å; F: 243-232 Å; G: 232-223 Å) are those previously identified by Krauss and Burke (2) based on frequency of occurence of specific LDL sizes in multicomponent GGE patterns of healthy subjects.

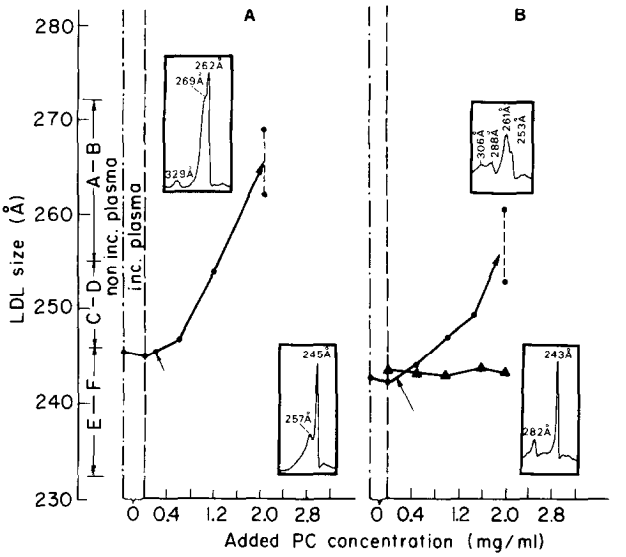


Figure 1A. Change in particle diameter of LDL from plasma (●—●) of subject EB as a function of added PC. Thin arrows in both A and B indicate threshold PC concentrations. Lower inset: GGE pattern (2/16 gel) of LDL from noninc (nonincubated) plasma; upper inset: GGE pattern (2/16 gel) of LDL from inc (incubated) plasma + 2.13 mg/ml PC; an additional minor peak (329 Å) is also observed at this PC level.

1B. Change in particle diameter of LDL from plasma (●—●) and from d < 1.20 g/ml fraction (▲—▲) of subject JG as a function of added PC. Lower inset: GGE pattern (2/16 gel) of LDL from noninc plasma; upper inset: GGE pattern (2/16 gel) of LDL from inc plasma + 2.0 mg/ml PC. The component with size 282 Å (probably Lp(a); lower inset) also increases in size with increasing PC levels (upper inset).

(0.2 mg/ml (EB) and 0.17 mg/ml (JG)), no change in LDL size was observed. In the PC range of 0.2-1.2 mg/ml (EB) and 0.17-1.5 mg/ml (JG), a progressive increase in LDL size occurred. A bimodal LDL pattern was observed at higher PC concentrations in both EB (2.1 mg/ml PC; Fig. 1A, upper inset) and JG (2.0 mg/ml PC; Fig. 1B, upper inset). Whereas the component of smaller size was the major contributor to the bimodal distribution of EB's LDL, the larger size component was the major contributor to the bimodal pattern of JG's LDL. At a still higher PC concentration (2.4 mg/ml) the bimodal pattern of EB's LDL was transformed into a single peak (272 Å; size group A). Thus, during incubation of plasma with increasing PC concentrations, major LDL subpopulations initially within size groups E or F were progressively converted to species within size groups C or D, and finally to species within size groups A or B.

To investigate whether the above PC-induced effects on major LDL species within size groups E and F would be observed for major LDL species of larger size (e.g.,

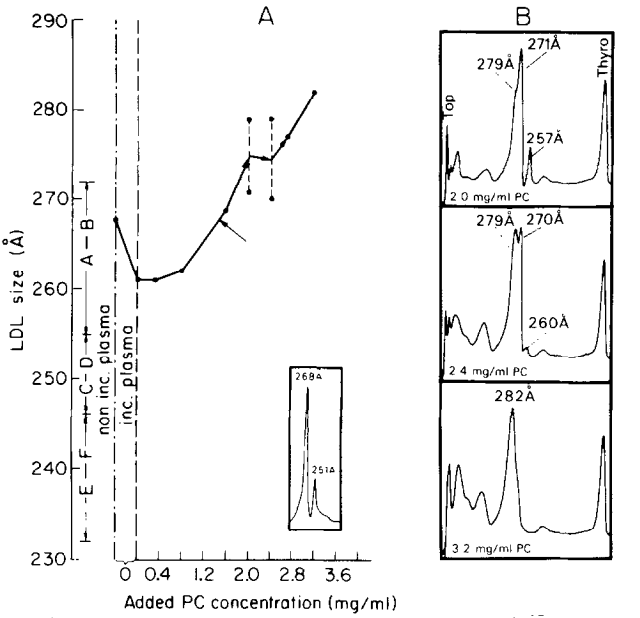


Figure 2A. Change in particle diameter of LDL from plasma (●—●) of subject RM as a function of added PC. The thin arrow points to the threshold PC concentration. Inset: GGE pattern (2/16 gel) of LDL from noninc plasma.

2B. GGE patterns of LDL from plasma (subject RM) incubated with increasing amounts of PC. Upper: 2.0 mg/ml; middle: 2.4 mg/ml; lower: 3.2 mg/ml. Gel top and peak of thyroglobulin standard (added to all samples just prior to GGE) are also indicated. Additional components larger in size than LDL are observed at these PC concentrations.

size groups A or B), plasma from subject RM, with a major LDL component of size 268 Å (size group A) and a minor component of size 251 Å (size group C) was studied. Incubation of plasma alone resulted in a shift of both LDL subpopulations towards smaller size (Fig. 2). We have consistently observed such decreases in LDL size (approximately 4-7 Å), upon incubation of plasma with major LDL species within size groups A or B. Inhibition of LCAT activity by PCMPS prevents such changes in LDL size, and hence the observed decreases in particle size may reflect reduction in the content of LDL surface components (PL, UC) brought about by LCAT activity during plasma incubation (14). Indeed, PC addition prevented the size decrease in RM's LDL brought about by plasma incubation (Fig. 2); this occurred at a threshold PC concentration of approximately 1.5 mg/ml. At PC levels above the threshold, changes in LDL properties similar in character to those noted in EB and JG could be distinguished. Thus, in the range between the threshold PC level and 2.0 mg/ml PC, LDL particle size appeared to increase. With increasing PC concentrations ($\geq$2.0 mg/ml), a bimodal pattern was observed as part of a

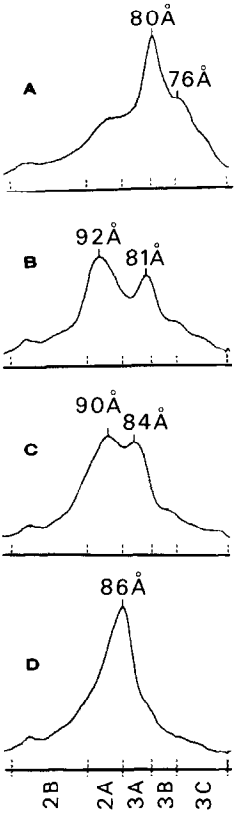


Figure 3. GGE patterns (4/30 gels) of HDL from EB's plasma. A, noninc plasma; B, inc plasma; C, inc plasma + 0.61 mg/ml PC; D, inc plasma + 1.22 mg/ml PC. HDL subpopulations, using nomenclature of Blanche et al. (1), are also indicated.

conversion sequence. First, a shoulder developed on the major LDL peak in the GGE pattern (Fig. 2, upper); this was followed by build-up of the shoulder, leading to a clearly bimodal profile (Fig. 2, middle); and, lastly, the bimodal profile was converted into a single major peak (Fig. 2, lower). Thus it appears that the formation of the bimodal LDL pattern, with increasing PC levels, is a characteristic of both large and small major LDL subpopulations.

Since PC uptake of HDL has been previously shown to occur under the conditions used in our present plasma incubations, we investigated whether there was any correlation between the changes in LDL patterns described above and those occurring in corresponding HDL patterns. Incubation of plasma (subject EB) in the absence of added PC resulted in redistribution of $(HDL_3)^2_{gge}$ material (Fig. 3A) towards larger

2. The terms $(HDL_3)_{gge}$, $(HDL_{2a})_{gge}$, etc. designate HDL species within particle size intervals identified by GGE which correspond approximately to HDL subclasses defined by ultracentrifugation (15).

species (predominantly $(HDL_{2a})_{gge}$; Fig. 3B), as previously reported (3). This led to a bimodal HDL pattern of components with diameters of 81 Å ($(HDL_{3a})_{gge}$) and 92 Å ($(HDL_{2a})_{gge}$). Addition of PC (0.61 mg/ml; Fig. 3C) gave a pattern with a larger $(HDL_{3a})_{gge}$ (84 Å) but a smaller $(HDL_{2a})_{gge}$ (90 Å) component. At 1.22 mg/ml PC (Fig. 3D), the HDL pattern showed only a single component (86 Å) which was predominantly within the $(HDL_{2a})_{gge}$ size region; above this PC concentration (2.13 mg/ml), the HDL pattern was comparable to that observed at 1.22 mg/ml. Similar results were obtained for HDL in JG's plasma. Other studies (7) have shown that incubation of isolated HDL with increasing PC levels results in a progressive increase in HDL size. Whereas $(HDL_3)_{gge}$ in incubated plasma also increases in size with PC, the $(HDL_{2a})_{gge}$, produced during plasma incubation without added PC, decreases in size and merges with $(HDL_{3a})_{gge}$ to form a single component predominantly within $(HDL_{2a})_{gge}$ region. Our data indicate that the PC-induced bimodal LDL pattern is observed only at PC concentrations above those where HDL converges to a single component (Fig. 3D). Such HDL interconversion requires PC uptake (6) and hence may influence the amount of PC available for LDL interconversion. In a similar manner, HDL may also influence the PC concentration at which the threshold for LDL size increase is observed. Thus, in plasma with substantial amounts of HDL (e.g., subject RM) the higher threshold PC concentration may reflect less PC available for interaction with LDL, due to PC uptake by HDL.

Since plasma contains a variety of proteins facilitating interconversion (3,16) and lipid transfer (17,18), we investigated whether removal of these proteins from plasma by ultracentrifugation (at d 1.21 g/ml) would prevent the PC-induced changes in LDL size. Figure 1B shows that incubation of the total lipoprotein fraction ($d < 1.20$ g/ml; subject JG), remaining after ultracentrifugation of plasma, with amounts of PC used in the whole plasma incubations, produced no increase in LDL size. These results indicated a requirement for some factor(s) in the $d > 1.20$ g/ml fraction. Incubation of plasma (all subjects) in the presence of PC (less than 2 mg/ml) with or without an LCAT inhibitor (PCMPS)[3], resulted in similar size increases in LDL. Preliminary chemical analyses showed no increase in LDL CE/protein

3. More than 98% of LCAT activity was inhibited by PCMPS in our experiments.

weight ratio (subjects RM and EB) as a function of PC concentration, suggesting that the LDL size increase was apparently not due to an LCAT-associated increase in core CE content. On the other hand, these analyses indicated an increase in LDL PL/protein weight ratio as a function of added PC concentration.[4] It is possible that a PL-transfer protein in plasma is involved in promoting PL uptake and subsequent size increase of LDL. Net uptake of PL from DPPC vesicles by LDL has been shown to occur in the presence of a PL-transfer protein (19).

Of interest are the potential physiological implications of our observations which indicate that PC in the presence of plasma factors can convert the particle size distributions of LDL (232-246 Å; size groups E-F) and HDL (72-88 Å; $HDL_3$) typically characteristic of hypertriglyceridemic subjects (20), to those representative of LDL (255-272 Å; size groups A-B) and HDL (enriched in $HDL_2$ species of 88-120 Å) encountered in plasma of normolipoproteinemic females (2,21). Plasma levels of higher $S_f^\circ$ rate (larger) LDL species in healthy subjects have been shown to correlate strongly with those of higher $F^\circ_{1.20}$ rate (larger) HDL species (21). In view of our studies, the particle size distributions of both LDL and HDL may be determined, in part, by the amount of PC available for interaction with these lipoprotein classes. Normalization of the chemical composition of PC-interconverted LDL would require additional modification of the LDL.

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